Monitoring Antihypertensive Medication Adherence by Liquid Chromatography–Tandem Mass Spectrometry: Method Establishment and Clinical Application

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Abstract: Proper medication compliance is critical for the integrity of clinical practice, directly related to the success of clinical trials to evaluate both pharmacological-based and device-based therapies. Here, we established a liquid chromatography–tandem mass spectrometry method to accurately detect 55 chemical entities in the human urine sample, which accounting for the most commonly used 172 antihypertensive drugs in China. The established method had good accuracy and intraday and interday precision for all analyses in both bench tests and validated in 21 hospitalized patients. We used this method to monitor and ensure drug compliance and exclude the inferring impacts of medication compliance as a key confounder for our pivotal trial of a catheter-based, renal mapping and selective renal denervation to treat hypertension. It is found that in the urine samples from 92 consecutive subjects, 85 subjects (92.4%) were consistent with their prescriptions after 28 days run-in periods, 90 (97.8%) and 85 (95.5%) patients completely complied with their medications during the 3-month and 6-month follow-up period, respectively. Thus, using the liquid chromatography–tandem mass spectrometry method with specificity, accuracy, and precision, we ensured drug compliance of patients, excluded the key confounder of drug interferences, and ensured the quality of our device-based clinical trial for treatment of hypertension.

Key Words: LC-MS/MS, medication compliance, hypertension, renal denervation

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INTRODUCTION

Medication adherence is defined as a patient's compliance with prescribed drugs during the period of treatment for their diseases.[1] The high proportion of poor medication compliance is a major challenge in clinical practice, where the average medication compliance for patients who suffer from chronic diseases is approximately 50%.[2] A typical example is hypertension, and this manifestation is a major risk factor for cardiovascular diseases such as stroke, coronary artery disease, and heart failure. Patients with hypertension need to take antihypertensive drugs throughout their life span. However, it has been reported that half of these patients discontinue their medication after 6 months of their first dose.[3] The poor medication compliance directly diminishes the control rate of hypertension.[4,5] For instance, at least 20%–35% of all hypertensive patients have not controlled their blood pressure properly because of this reason,[6–8] resulting in increased incidences of cardiovascular morbidity and mortality. This issue is particularly severe in China. It has been reported that among 245 million patients with hypertension,[9] the controlled rate is only 6% because of poor medication compliance.[10]

The importance of drug compliance is also directly related to the success of clinical trials to evaluate both pharmacological-based[1] and device-based therapies.[11] Investigators usually assume the subject takes the tested drugs as prescribed, but in reality, it is difficult for the subject to take full doses of the tested drug on time and achieve expected efficacy according to trial protocols. The overall medication compliance of patients in drug clinical trials varied between 43% and 78%.[12,13] In a clinical trial of an antihypertensive drug, subjects with poor adherence will inevitably produce type II statistical errors, making it more difficult to judge the therapeutic effects, especially for drugs with narrow treatment windows.[14] These issues of poor drug compliance also appear in clinical trials of nonpharmacological antihypertensive therapies. When subjects are treated with a device to lower their blood pressure while simultaneously taking antihypertensive drugs during clinical trials, drug compliance is a crucial confounding factor and interferes with the effects of the device on blood pressure, which may ultimately overturn clinical trial conclusions. We have been facing this particular challenge during a clinical trial for a novel device therapy to treat hypertension.[15]

Renal denervation (RDN) is a catheter-based device therapy to remove renal sympathetic nerves using energies such as radiofrequency (RF) or ultrasound[16] and is a hotly pursued approach for treatment of hypertension in recent years.

We have developed a renal nerve mapping and RF ablation system for selectively denervating renal sympathetic nerves. A pivotal trial for the system is currently being conducted (www.clinicaltrials.gov identifier NCT02761811). A key element of the study is to monitor antihypertensive drug compliance and exclude the inferring impacts of the confounders on the device-induced blood pressure reductions. To address this issue, a reliable approach is needed. There are several existing methods for monitoring medication adherence, including patient self-report, drug tablet counting, prescription tracking, and an electronic medication monitoring system.[17] These methods are affected by various factors, such as the behavior of patients and clinicians or the complexity of clinical practice.[1] Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is one of the most reliable ways to address this issue.

However, the efforts to establish the LC-MS/MS methods are a great challenge in the clinical environment in China because so many generic antihypertensive drugs are being used by a large hypertensive patient population, yet there is a very low drug compliance among those patients in China. Numerous generic drugs are being used in clinical practice in China, where there are on average 113.9 approved drugs for each active ingredient, whereas the US FDA has an average of only 4.2 approved drugs per active ingredient.[18] In this study, we surveyed representative medical university affiliated hospitals regarding how many commercially available antihypertensive drugs they use and how many chemical entities are covered by these drugs; we established a LC-MS/MS method to accurately detect the most commonly used antihypertensive drugs by patients in China and used this method to ensure drug compliance during our catheter-based therapy for treatment of hypertension.

MATERIALS AND METHODS

Survey of Antihypertensive Drugs and Determination of Chemical Entities Library

To determine which compounds should be collected into our library for LC-MS/MS analysis, we sent out a survey sheet to 13 hospitals, which are listed in Table 1, and investigated how many hypertensive drugs are currently used in mainstream clinical practice in China. These hospitals include medical university affiliated teaching hospitals located in 8 provincial capital cities, representative of the current range of prescription options for hypertensive patients in China.

Based on our survey, there were surprisingly 172 antihypertensive drugs used by these hospitals in clinical practice. These drugs present 55 chemical compounds and 6 classes of antihypertensive drugs. These drugs, the manufacturers, and corresponding chemical entities are summarized in Table 2.

Sources of Reagents and Standards

To establish methods for examination of these compounds in clinical samples by LC-MS/MS, methanol and dimethyl sulfoxide with chromatographic purity were purchased from Sigma (St Quentin-Fallavier, France) and formic acid with analytical purity was from Merck (VWR, Fontenay sous Bois, France). Purified water was obtained from Mili-Q, 18.2 MΩ cm (Millipore, Bedfore). The standards and commercial sources of standards for LC-MS/MS are listed in Table 3.

TABLE 1. Survey of Antihypertensive Drugs in 13 Hospitals

1	Peking University First Hospital, Beijing, China
2	Peking University People's Hospital, Beijing, China
3	Peking University Third Hospital, Beijing, China
4	The First Affiliated Hospital with Nanjing Medical University, Nanjing, China
5	Renmin Hospital of Wuhan University, Wuhan, China
6	The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China
7	The 306th Hospital of the Liberation Army, Tianjin, China
8	Tianjin Affiliated Hospital of Armed Police Medical College
9	Tianjin First Central Hospital, Tianjin, China
10	Shanghai Jiaotong University School of medicine Renjin Hospital, Shanghai, China
11	Bethune International Peace Hospital, Shijiazhuang, China
12	Hebei General Hospital, Shijiazhuang, China
13	Sichuan University West China Hospital, Chengdu, China

Apparatus and Conditions

The analysis processes were performed through a Shimadzu 20A HPLC system (Shimadzu Kyoto, Japan) equipped with 4 modules: Nexera XR LC-20AD liquid pump, SIL-20AC autosampler, CTO-20AC column oven, and DGU-20A degasser. The mass spectrometric detector was an API 4000 triple-quadrupole mass spectrometer (SCIEX, Ontario, Canada). The acquisition and data analysis software (version 1.6.3) were supplied by SCIEX (Framingham MA). Several internal standards were used for positive mode compound quantitation, and glipizide was used for negative mode quantitation except furosemide quantitation, which is an external standard calibration, was used. These internal and external standards are listed in Table 3.

Two analytical methods were used in the current study: positive and negative ion detection methods. Forty-nine compounds were detected by the former, and 6 compounds were detected by the latter. The mass spectrometer conditions of these 2 detection methods are briefly described below.

Liquid Chromatography–Tandem Mass Spectrometry Conditions for Positive Ion Detection

The devices and parameters of separation using a gradient elution by reversed-phase chromatography are listed below.

Column: Agilent Eclipse XDB-C18 (4.6 × 150 mm, 5 μm); column temperature: 40°C; mobile phase A: 0.1% formic acid–water; mobile phase B: 0.1% formic acid–methanol; flow rate: 0.800 mL/min (no split); and injection volume: 5 μL.

Gradient:

Time, min	0	1.2	3	6.5	8.5	8.6	10
B%	10	10	60	95	95	10	10

TABLE 2. One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
Angiotensin-converting enzyme inhibitors (ACEI): 18 drugs				
1	ACEI	ACERTIL	Perindopril tablets	Servier (Tianjin) Pharmaceutical Co, Ltd
2	ACEI	AILIYA	Enalapril maleate dispersible tablet	Shandong Luyin Pharmaceutical Co, Ltd
3	ACEI	CAPOTEN	Captopril tablets	Sino-American Shanghai Squibb Pharmaceutical Co, Ltd
4	ACEI	CHANGYAO	Captopril tablets	Changzhou Pharmaceutical Factory Co, Ltd
5	ACEI	DASHUANG	Imidapril hydrochloride tablets	Tianjin Tanabe Pharmaceutical Co, Ltd
6	ACEI	FUTIANLE	Enalapril maleate capsules	Actavis (Foshan) Pharmaceutical Co, Ltd
7	ACEI	LOTENSIN	Benazepril hydrochloride tablets	Beijing Novartis Pharmaceutical Co, Ltd
8	ACEI	MONOPRIL	Fosinopril sodium tablets	Sino-American Shanghai Squibb Pharmaceutical Co, Ltd
9	ACEI	RENITEC	Enalapril maleate tablets	Hangzhou Merck Pharmaceutical Co, Ltd
10	ACEI	ROTAM	Ramipril tablets	Kunshan Longdeng Ruidi Pharmaceutical Co, Ltd
11	ACEI	SHYNPEC	Enalapril maleate tablets	Shanghai Modern Pharmaceutical Co, Ltd
12	ACEI	TRITACE	Ramipril tablets	Sanofi (Beijing) Pharmaceutical Co, Ltd
13	ACEI	XINDAYI	Benazepril hydrochloride tablets	Shenzhen Xinlitai Pharmaceutical Co, Ltd
14	ACEI	XINYAFUSHU	Benazepril hydrochloride tablets	Shanghai Xinya Pharmaceutical Minhang Co, Ltd
15	ACEI	YALI	Fosinopril sodium tablets	Zhejiang Huahai Pharmaceutical Co, Ltd
16	ACEI	YIHENG	Quinapril hydrochloride tablets	Harbin Pharmaceutical Group Pharmaceutical General Factory
17	ACEI	YISU	Enalapril maleate tablets	Yangtze River Pharmaceutical Group Jiangsu Pharmaceutical Co, Ltd
18	ACEI	ZESTRIL	Lisinopril tablets	AstraZeneca UK Limited
Angiotensin II receptor blockers (ARB): 29 drugs				
19	ARB	ANNEIQIANG	Telmisartan Tablets	Suzhou Dawnrays Pharmaceutical Co, Ltd
20	ARB	AOBIXIN	Candesartan cilexetil dispersible tablets	Kunming Yuanrui Pharmaceutical Co, Ltd
21	ARB	APROVEL	Irbesartan tablets	Sanofi (Hangzhou) Pharmaceutical Co, Ltd
22	ARB	BANGTAN	Telmisartan tablets	Jiangsu Wanbang Biochemical Pharmaceutical Co, Ltd
23	ARB	BEIYI	Losartan potassium tablets	Zhejiang Huahai Pharmaceutical Co, Ltd
24	ARB	BLOPRESS	Candesartan cilexetil tablets	Tianjin Takeda Pharmaceutical Co, Ltd
25	ARB	BOLIGAO	Candesartan cilexetil tablets	Zhejiang Yongning Pharmaceutical Co, Ltd
26	ARB	COZAAR	Losartan potassium tablets	Hangzhou Merck Pharmaceutical Co, Ltd
27	ARB	DIOVAN	Valsartan capsules	Beijing Novartis Pharmaceutical Co, Ltd
28	ARB	GEPING	Irbesartan tablets	Xiuzheng Pharmaceutical Group Co, Ltd
29	ARB	HUIYUE	Valsartan capsules	Beijing Secco Pharmaceutical Co, Ltd
30	ARB	JIJIA	Irbesartan tablets	Jiangsu Hengrui Pharmaceutical Co, Ltd
31	ARB	KESU	Irbesartan tablets	Yangtze River Pharmaceutical Group Beijing Haiyan Pharmaceutical Co, Ltd
32	ARB	LIWEN	Telmisartan tablets	Hainan Seric Pharmaceutical Co, Ltd
33	ARB	MICARDIS	Telmisartan tablets	Shanghai Boehringer Ingelheim Pharmaceutical Co, Ltd
34	ARB	MILETAN	Telmisartan capsules	Shanxi Huayuan Pharmaceutical Biotechnology Co, Ltd

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TABLE 2. (*Continued*) One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
35	ARB	NILIAN	Candesartan cilexetil capsules	Qingdao Huanghai Pharmaceutical Co, Ltd
36	ARB	NUOJINPING	Telmisartan tablets	Zhejiang Jinliyuan Pharmaceutical Co, Ltd
37	ARB	OLMETEC	Olmesartan medoxomil tablets	Daiichi Sankyo Pharmaceutical (Shanghai) Co, Ltd
38	ARB	OUMEINING	Telmisartan tablets	Yichang East Sunshine Yangtze River Pharmaceutical Co, Ltd
39	ARB	SHUNIYA	Telmisartan capsules	Beijing Fuyuan Pharmaceutical Co, Ltd
40	ARB	SUIYUE	Valsartan capsules	China Resources SECCO Pharmaceutical Co, Ltd
41	ARB	WEIERYA	Candesartan cilexetil tablets	Chongqing Shenghuaxi Pharmaceutical Co, Ltd
42	ARB	XIEKE	Valsartan capsules	Changzhou Siyao Pharmaceutical Co, Ltd
43	ARB	XINJUNNING	Candesartan cilexetil tablets	Guangzhou Baiyunshan Tianxin Pharmaceutical Co, Ltd
44	ARB	YIDALE	Valsartan dispersible tablets	Hainan Huanglong Pharmaceutical Co, Ltd
45	ARB	YIFANG	Valsartan capsules	Hainan Aomeihua Pharmaceutical Co, Ltd
46	ARB	YITAIQING	Irbesartan capsules	Zhuhai Rundu Pharmaceutical Co, Ltd
47	ARB	YULENING	Telmisartan tablets	Suzhou Sinochem Pharmaceutical Industry Co, Ltd
Calcium channel blockers (CCB): 38 drugs				
48	CCB	ADALAT	Nifedipine controlled released tablets	Bayer Healthcare Co, Ltd
49	CCB	ANNEIZHEN	Amlodipine besylate tablets	Suzhou Dawnrays Pharmaceutical Co, Ltd
50	CCB	BILUOPING	Amlodipine besylate tablets	Guangdong Pidi Pharmaceutical Co, Ltd
51	CCB	CONIEL	Benidipine hydrochloride tablets	Kyowa Fermentation Kirin Co, Ltd
52	CCB	DEGAONING	Nifedipine sustained-release tablets	Dezhou Deyao Pharmaceutical Co, Ltd
53	CCB	DIAO	Extended release nifedipine tablets	Diao Group Chengdu Pharmaceutical Co, Ltd
54	CCB	ETERSIM	Felodipine sustained-release tablets	Nanjing Yiheng Pharmaceutical Co, Ltd
55	CCB	HEBEI-SHUANG	Diltiazem hydrochloride sustained-release capsules	Tianjin Tanabe Pharmaceutical Co, Ltd
56	CCB	HEXIN-SHUANG	Diltiazem hydrochloride tablets	Tianjin Tanabe Pharmaceutical Co, Ltd
57	CCB	HUANANPAI	Nimodipine tablets	Guangdong Huanan Pharmaceutical Group Co, Ltd
58	CCB	HUANANPAI	Nitrendipine tablets	Guangdong Huanan Pharmaceutical Group Co, Ltd
59	CCB	HUANANPAI	Verapamil hydrochloride tablets	Guangdong Huanan Pharmaceutical Group Co, Ltd
60	CCB	ISOPTIN SR	Verapamil hydrochloride SR tablets	AbbVie Deutschland GmbH and Co. KG
61	CCB	JIBEIER	Nitrendipine and atenolol tablets	Jiangsu Jibeier Pharmaceutical Co, Ltd
62	CCB	KANGBAO-DEWEI	Felodipine sustained-release tablets	Shanxi Kangbao Biological Products Co, Ltd
63	CCB	LACIPIL	Lacidipine tablets	GLAXOSMITHKLINE, S.A.
64	CCB	LANDI	Amlodipine besylate tablets	Yangtze River Pharmaceutical Group Shanghai Haini Pharmaceutical Co, Ltd
65	CCB	LIANHUAN-XIAODING	Felodipine sustained-release capsules	Jiangsu Lianhuan Pharmaceutical Co, Ltd

TABLE 2. (*Continued*) One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
66	CCB	LIFANGLINUO	Felodipine sustained-release tablets	Hefei Lifang Pharmaceutical Co, Ltd
67	CCB	LISIDE	Aspartic acid amlodipine tablets	Zhejiang Jianfeng Pharmaceutical Co, Ltd
68	CCB	NIFUDA	Nifedipine sustained-release tablets	Qingdao Huanghai Pharmaceutical Co, Ltd
69	CCB	NILISU	Nimodipine injection	Shandong Xinhua Pharmaceutical Co, Ltd
70	CCB	NIMOTOP	Nimodipine tablets	Bayer Healthcare Co, Ltd
71	CCB	NORVASC	Amlodipine besylate tablets	Pfizer Pharmaceutical Co, Ltd
72	CCB	PERDIPINE	Nicardipine hydrochloride sustained-release capsules	Astellas Pharmaceutical (China) Co, Ltd
73	CCB	PLENDIL	Felodipine sustained-release tablets	AstraZeneca Pharmaceutical Co, Ltd
74	CCB	SHENG TONG-PING	Nifedipine sustained-release tablets	Sinopharm Guangdong Global Pharmaceutical Co, Ltd
75	CCB	SIBELIUM	Flunarizine hydrochloride capsules	Xi'an Janssen Pharmaceutical Co, Ltd
76	CCB	XINGAIDA	Nifedipine sustained-release tablets	Yantai Luyin Pharmaceutical Co, Ltd
77	CCB	XINHAINING	Amlodipine besylate tablets	Yichang East Sunshine Yangtze River Pharmaceutical Co, Ltd
78	CCB	XINRAN	Nifedipine controlled released tablets	Shanghai Modern Pharmaceutical Co, Ltd
79	CCB	XINTONG-DINGPIAN	Nifedipine tablets	Tianjin Pacific Pharmaceutical Co, Ltd
80	CCB	YASHIDA	Amlodipine besylate tablets	China Resources SECCO Pharmaceutical Co, Ltd
81	CCB	YASIKEPING	Amlodipine besylate tablets	Ningbo Dahongying Pharmaceutical Co, Ltd
82	CCB	YIBODING	Verapamil hydrochloride tablets	Jiangsu Ruinian Qianjin Pharmaceutical Co, Ltd
83	CCB	YIFULIN	Nimodipine sustained-release tablets	Qilu Pharmaceutical Co, Ltd
84	CCB	YUANZHI	Benidipine hydrochloride tablets	Shandong Huasu Pharmaceutical Co, Ltd
85	CCB	ZANIDIP	Lercanidipine hydrochloride tablets	Recordati S.P.A. (Italy)
β-adrenoceptor antagonists (β-blockers): 14 drugs				
86	β-blocker	AILUO	Esmolol hydrochloride injection	Qilu Pharmaceutical Co, Ltd
87	β-blocker	ALMARL	Arotinolol hydrochloride tablets	Dainippon Sumitomo Pharma Co, Ltd. Ibaraki Plant (Japan)
88	β-blocker	ANXIAN-XINAN	Atenolol tablets	Tianjin Central Pharmaceutical Co, Ltd
89	β-blocker	BETALOC	Metoprolol tartrate tablets	AstraZeneca Pharmaceutical Co, Ltd
90	β-blocker	BETALOC SR	Metoprolol succinate sustained-release tablets	AstraZeneca AB (Sweden)
91	β-blocker	BOSU	Bisoprolol fumarate tablets	Beijing Huasu Pharmaceutical Co, Ltd
92	β-blocker	CONCOR	Bisoprolol fumarate tablets	Merck KGaA (Merck, Germany)
93	β-blocker	DILATREND	Carvedilol tablets	Shanghai Roche Pharmaceutical Co, Ltd
94	β-blocker	DISAINUO	Labetalol hydrochloride tablets	Jiangsu Desano Pharmaceutical Co, Ltd
95	β-blocker	JINLUO	Carvedilol tablets	Qilu Pharmaceutical Co, Ltd
96	β-blocker	KANGDAXIN	Carvedilol tablets	Chongqing Huachuang Pharmaceutical Co, Ltd
97	β-blocker	SONGSHU	Atenolol tablets	Tianjin Central Pharmaceutical Co, Ltd
98	β-blocker	WEITE	Sotalol hydrochloride tablets	Lunanbet Pharmaceutical Co, Ltd

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TABLE 2. (*Continued*) One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
99	β-blocker	XINDEAN	Propranolol hydrochloride tablets	Shantou Jinshi Pharmaceutical General Factory
α-adrenoceptor antagonists (α-blockers): 15 drugs				
100	α-blocker	CARDURA	Doxazosin	Pfizer Pharmaceutical Co, Ltd
101	α-blocker	HYTRIN	Terazosin hydrochloride tablets	Shanghai Abbott Pharmaceutical Co, Ltd
102	α-blocker	LUOHAO	Urapidil tablets	Shandong Luoxin Pharmaceutical Group Co, Ltd
103	α-blocker	LUOXINPING	Doxazosin mesylate tablets	Kangmei Pharmaceutical Co, Ltd
104	α-blocker	LIXIDING	Urapidil sustained-release tablets	Xi'an Lijun Pharmaceutical Co, Ltd
105	α-blocker	MASHANI	Terazosin hydrochloride tablets	China Resources SECCO Pharmaceutical Co, Ltd
106	α-blocker	OUDEMAN	Terazosin hydrochloride capsules	Chongqing Watson Pharmaceutical Co, Ltd
107	α-blocker	REGITINE	Phentolamine	Nycomed Austria GmbH
108	α-blocker	RUITONG	Alfuzosin hydrochloride tablets	Shenyang Tianling Pharmaceutical Co, Ltd
109	α-blocker	TAILE	Terazosin hydrochloride capsules	Yangtze River Pharmaceutical Group Jiangsu Pharmaceutical Co., Ltd
110	α-blocker	WEIPING	Alfuzosin hydrochloride tablets	Lunanbet Pharmaceutical Co, Ltd
111	α-blocker	XATRAL	Alfurazosin hydrochloride sustained-release tablets	Sanofi (Hangzhou) Pharmaceutical Co, Ltd
112	α-blocker	XINYI	Prazosin hydrochloride tablets	Shanghai Xinyi Pharmaceutical Co, Ltd
113	α-blocker	YANINGDING	Urapidil tablets	Altana Pharma AG
114	α-blocker	YONGHE	Methyldopa tablets	Zhengzhou Yonghe Pharmaceutical Co, Ltd
Diuretics: 17 drugs				
115	Diuretic	CHAN GYAO	Hydrochlorothiazide tablets	Changzhou Pharmaceutical Factory Co, Ltd
116	Diuretic	GUANGHUI	Furosemide tablets	Shanghai Zhaohui Pharmaceutical Co, Ltd
117	Diuretic	HAIWANG	Bumetanide tablets	Fuzhou Neptunus Pharmaceutical Co, Ltd
118	Diuretic	KANGHE	Spironolactone tablets	Guangzhou Kanghe Pharmaceutical Co, Ltd
119	Diuretic	LIZHI	Torasemide capsules	Zhejiang Chengyi Pharmaceutical Co, Ltd
120	Diuretic	LUQUAN	Torasemide injection	Zhejiang Chengyi Pharmaceutical Co, Ltd
121	Diuretic	NATRILIX	Indapamide sustained-release tablets	Servier (Tianjin) Pharmaceutical Co, Ltd
122	Diuretic	SHOUBI-SHAN	Indapamide tablets	Tianjin Lisheng Pharmaceutical Co, Ltd
123	Diuretic	SHUANGHE	Bendroflumethiazide tablets	China Resources Double-Crane Pharmaceutical Co, Ltd
124	Diuretic	SHUANGHE	Chlorthalidone tablets	China Resources Double-Crane Pharmaceutical Co, Ltd
125	Diuretic	TESUNI	Torasemide	Nanjing Youke Pharmaceutical Co, Ltd
126	Diuretic	TUOSAI	Torasemide tablets	Jiangsu Suzhong Pharmaceutical Group Co, Ltd
127	Diuretic	XINYI	Spironolactone tablets	Shanghai Xinyi Pharmaceutical Co, Ltd
128	Diuretic	YIMAIGE	Torasemide tablets	Hubei Encyclopedia Hendy Pharmaceutical Co, Ltd
129	Diuretic	YITEAN	Indapamide tablets	Zhejiang Prokangyu Pharmaceutical Co, Ltd
130	Diuretic	YUENAN-SHAN	Indapamide SR capsules	Tianjin Pacific Pharmaceutical Co, Ltd
131	Diuretic	ZETONG	Torsemide for injection	Nanjing Haichen Pharmaceutical Co, Ltd

TABLE 2. (*Continued*) One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
Vasodilators/nitrates: 7 drugs				
132	Vasodilator	AIBEI	Isosorbide dinitrate injection	Qilu Pharmaceutical Co, Ltd
133	Vasodilator	DOUBLE -CRANE	Sodium nitroprusside for injection	China Resources Double-Crane Pharmaceutical Co, Ltd
134	Vasodilator	IMDUR	Isosorbide mononitrate sustained-release tablets	AstraZeneca Pharmaceutical Co, Ltd
135	Vasodilator	JINGYI	Nitroglycerin tablets	Beijing Yimin Pharmaceutical Co, Ltd
136	Vasodilator	XINKANG	Isosorbide mononitrate tablets	Lunanbet Pharmaceutical Co, Ltd
137	Vasodilator	YILEDING	Isosorbide mononitrate sustained-release capsules	UCB (Zhuhai) Pharmaceutical Co, Ltd
138	Vasodilator	YISHUJI	Isosorbide dinitrate spray	UCB (Zhuhai) Pharmaceutical Co, Ltd
Others agents: 3 drugs				
139	Others	Leiyunshang	Hydralazine hydrochloride tablets	Guangdong Leiyunshang Pharmaceutical Co, Ltd
140	Others	RASILEZ	Aliskiren tablets	Novartis Farma S.p.A.
141	Others	YANIDING	Moxonidine hydrochloride tablets	Shanghai Renhu Pharmaceutical Co, Ltd
Compound drugs: 31 drugs				
142	Compound	ANLIBO	Irbesartan and hydrochlorothiazide capsules	Yuanhe Pharmaceutical Co, Ltd
143	Compound	ANNEIXI	Losartan potassium and hydrochlorothiazide tablets	Suzhou Dawnrays Pharmaceutical Co, Ltd
144	Compound	ANWEILE	Irbesartan and hydrochlorothiazide capsules	Beijing Sihuan Kebao Pharmaceutical Co, Ltd
145	Compound	BAIANXIN	Amlodipine and benazepril tablets	Yangtze River Pharmaceutical Group Guangzhou Hairui Pharmaceutical Co, Ltd
146	Compound	BEIJINGJ IANGYA NO.1	Compound hypotensive tablets	China Resources Double-Crane Pharmaceutical Co, Ltd
147	Compound	BEIYUE	Irbesartan and hydrochlorothiazide tablets	Zhejiang Huahai Pharmaceutical Co, Ltd
148	Compound	BILBOT	Valsartan and amlodipine	Beijing Novartis Pharmaceutical Co, Ltd
149	Compound	BIPREL	Perindopril tert-butylamine and indapamide	Servier (Tianjin) Pharmaceutical Co, Ltd
150	Compound	CHANGYAO-JIANGYA	Compound dihydralazine sulfate tablets	Changzhou Pharmaceutical Factory Co, Ltd
151	Compound	COAPROVEL	Irbesartan and hydrochlorothiazide tablets	Sanofi (Hangzhou) Pharmaceutical Co, Ltd
152	Compound	CO-DIOVAN	Valsartan and hydrochlorothiazide tablets	Novartis Farma S.p.A (Italy)
153	Compound	COMPOUND RESERPINE	Compound reserpine tablets	Yabao Pharmaceutical Group Co, Ltd
154	Compound	DUODAYI	Amlodipine and atorvastatin calcium tablets	Pfizer Pharmaceutical Co, Ltd
155	Compound	ESTEIN	Benazepril hydrochloride and hydrochlorothiazide tablets	Beijing Novartis Pharmaceutical Co, Ltd
156	Compound	GAOXUEYA-SUJIANG	Hypertension sujiang pill	Beijing Tongrentang Technology Development Co, Ltd Pharmaceutical Factory
157	Compound	HYZAAR	Losartan potassium and hydrochlorothiazide tablets	Hangzhou Merck Pharmaceutical Co, Ltd
158	β-blocker	JIBEIER	Nitrendipine and atenolol tablets	Jiangsu Jibeier Pharmaceutical Co, Ltd
159	Compound	JINVALIC	Valsartan and hydrochlorothiade tablets	Changzhou Siyao Pharmaceutical Co, Ltd
160	Compound	JIUBAOKE	Enalapril maleate and hydrochlorothiazide tablets	Beijing Honglin Pharmaceutical Co, Ltd
161	Compound	KAIFUTE	Compound captopril tablets	Changzhou Pharmaceutical Factory Co, Ltd

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TABLE 2. (*Continued*) One Hundred Seventy-Two Antihypertensive Drugs Used in 13 Representative Hospitals in China

No.	Class	Brand Name	Generic Name	Manufacturer
162	Compound	LIPEI	Irbesartan and hydrochlorothiazide dispersible tablets	Jiangsu Wangao Pharmaceutical Co, Ltd
163	Compound	MICARDIS	Telmisartan and hydrochlorothiazide tablets	Boehringer Ingelheim International GmbH
164	Compound	NAIDIYA	Losartan potassium and hydrochlorothiazide tablets	Beijing Fuyuan Pharmaceutical Co, Ltd
165	Compound	NIUHUANG-JIANGYA	Niuhuang antihypertensive pills	Beijing Tongrentang Technology Development Co, Ltd Pharmaceutical Factory
166	Compound	OLMETEC	Olmesartan medoxomil hydrochlorothiazide tablets	Daiichi Sankyo Pharmaceutical (Shanghai) Co, Ltd
167	Compound	QINGNAO-JIANGYA	Qingnao Jiangya tablets	haanxi Panlong Pharmaceutical Group Co, Ltd
168	Compound	WUDULI	Compound amiloride hydrochloride tablets	Jiangsu Desano Pharmaceutical Co, Ltd
169	Compound	YILUNPING	Irbesartan and hydrochlorothiazide tablets	Nanjing Zhengda Tianqing Pharmaceutical Co, Ltd
170	Compound	YIYE	Enalapril maleate and folic acid tablets	Shenzhen Osa Pharmaceutical Co, Ltd
171	Compound	YUANSITAN	Losartan potassium and hydrochlorothiazide tablets	Lepu Pharmaceutical Technology Co, Ltd
172	Compound	ZHENJV-JIANGYA	Zhenju antihypertensive tablets	Yabao Pharmaceutical Group Co, Ltd

Ion detection method: multiple reaction monitoring (MRM), ionization mode: electrospray ionization, ion polarity: positive ion, ionization voltage (IS): 5500 V, temperature: 550°C, gas 1: 60 psi, gas 2: 65 psi, curtain gas: 35 psi, and collision gas: 5 psi.

The chemical structures of 49 compounds, mass spectra, and detection ion pair m/z values, optimal declustering potential, and collision energy used by positive ion detection are shown in Table 3.

Liquid Chromatography–Tandem Mass Spectrometry Conditions for Negative Ion Detection

The devices and parameters using a gradient elution by reversed-phase chromatography were listed as below.

Column: Agilent Eclipse XDB-C18 (4.6 × 150 mm, 5 μm), column temperature: 40°C, mobile phase A: 0.1% formic acid–water, mobile phase B: 0.1% formic acid–methanol, flow rate: 0.800 mL/min (no split), and injection volume: 5 μL.

Gradient:

Time, min	0	1.2	4.5	8.5	10.5	10.6	13
B%	10	10	60	95	95	10	10

Ion detection method: MRM, ionization mode: electrospray ionization, ion polarity: negative ion, IS: −4500 V, temperature: 550°C, gas 1: 60 psi, gas 2: 65 psi, curtain gas: 30 psi, and collision gas: 5 psi.

The chemical structures of the 6 compounds, mass spectra, and the detection ion pair m/z values, optimal declustering potential and collision energy used by negative ion detection are shown in Table 3, where lacidipine, nitrendipine, hydrochloroyhiazide, furosemide, bendroflumethiazide, and chlorthalidone were the 6 antihypertensive agents.

Urine Sample Preparation for LC-MS/MS Analysis

Urine samples were collected from hospitals for LC-MS/MS analysis. Processes for protein precipitation were performed. In brief, 50 μL methanol was added to a 50 μL human urine sample, then 200 μL internal standard working solution (prepared in methanol) was added, vortexed for 5 minutes, and centrifuged at 14,000 rpm for 5 minutes. Milli-Q H_2O (50 μL) was added to 50 μL supernatant, vortexed for 2 minutes, and centrifuged at 14,000 rpm for 5 minutes, and the supernatant was injected to the LC-MS/MS system. After the processes, the urine sample did not contain endogenous substances that interfered with our analytical method. This blank urine was stored at −20°C and used as standard curve sample (STD) and quality control sample (QC).

Analysis Procedure

The prepared urine sample (50.0 μL) was placed in a labeled polypropylene (PP) tube and used for standard curves, QC, or blank samples. Methanol (50.0 μL) was added to the tubes containing urine samples, single blank sample tubes, and double blank sample tubes, respectively. Corresponding working solution (50.0 μL) was added to the tubes containing samples for standard curve and QC, respectively. These tubes were vortexed for 5 minutes, centrifuged at 14,000 rpm for 5 minutes, then 50.0 μL supernatant from each tube was transferred to another set of labeled PP tubes, and 50.0 μL of Milli-Q H_2O was added. These

TABLE 3. Fifty-five Antihypertensive Chemical Entities and Mass Spectrometry Parameters

No.	Antihypertensive Agents	Standard Manufacturer	Q1–Q3 (Da)	Declustering Potential (V)	Collision Energy (eV)	t_R, min	LLOQ, ng/mL	ULOQ, ng/mL	Internal Standard
ACEI									
1*	Benazeprilat (Benazapril Meta)	NA	397.0–351.0	105	30	7.44	1.00	1000	Ketoconazole
2	Captopril	Sigma Aldrich	218.1–116.1	73	20	6.18	20.0	20,000	Tiapride
3	Enalapril	Cayman Chemistry	377.2–234.1	70	26	6.67	1.00	1000	Tiapride
4	Fosinopril	Sigma Aldrich	564.4–492.2	84	11	10.71	20.0	20,000	Dexamethasone
5	Imidapril	Sigma Aldrich	406.2–234.0	75	27	6.86	1.00	1000	Ketoconazole
6	Lisinopril	Sigma Aldrich	406.2–84.0	78	52	5.24	5.00	5000	Tiapride
7	Perindopril	Fluka	369.1–172.1	90	31	6.96	1.00	1000	Ketoconazole
8	Quinapril	Fluka	439.1–234.0	90	27	7.66	1.00	1000	Ketoconazole
9	Ramipril	Sigma Aldrich	417.3–234.1	78	29	7.43	1.00	1000	Ketoconazole
ARB									
10	Candesartan	Aladdin	441.1–263.0	97	18	8.21	5.00	5000	Ketoconazole
11	Irbesartan	Sigma Aldrich	429.4–206.8	108	38	8.43	1.00	1000	Tiapride
12	Losartan	Fluka	423.2–207.0	69	33	8.07	1.00	1000	Ketoconazole
13	Olmesartan	Aladdin	447.1–207.0	83	37	6.60	5.00	5000	Ketoconazole
14*	Telmisartan glucuronide (Telmisartan Meta)	NA	691.4–515.4	80	20	7.86	5.00	5000	Ketoconazole
15	Valsartan	Sigma Aldrich	436.3–206.9	90	38	8.76	20.0	20,000	Ketoconazole
CCB									
16	Amlodipine	Sigma Aldrich	409.0–237.9	69	12	7.13	5.00	5000	Ketoconazole
17	Benidipine	NIFDC	506.2–174.0	78	37	6.80	1.00	1000	Ketoconazole
18*	Dehydrofelodipine (Felodipine Meta)	NA	384.0–320.0	70	20	9.80	50.0	50,000	Ketoconazole
19	Dehydronifedipine (Nifedipine Meta)*	NA	331.2–270.2	70	40	8.19	50.0	50,000	Ketoconazole
20	Diltiazem	Sigma Aldrich	415.1–178.0	85	37	6.61	1.00	1000	Tiapride
21	Flunarizine	Sigma Aldrich	405.2–203.0	79	21	7.65	1.00	1000	Ketoconazole
22	Lacidipine	Sigma Aldrich	454.1–408.0	−95	−23	8.38	1.00	1000	Glipizide
23	Lercanidipine	Sigma Aldrich	612.2–280.2	94	31	7.75	1.00	1000	Ketoconazole
24	Nicardipine	Sigma Aldrich	480.2–315.1	78	34	6.65	1.00	1000	Tiapride
25	Nimodipine	Sigma Aldrich	419.2–343.1	82	13	9.17	5.00	5000	Diclofenac
26	Nitrendipine	Sigma Aldrich	359.0–121.9	−86	−25	7.28	1.00	1000	Glipizide
27	Verapamil	Sigma Aldrich	455.3–165.0	111	41	6.59	1.00	1000	Ketoconazole
β-adrenoceptor antagonists (β-blockers)									
28	Arotinolol	Sigma Aldrich	372.0–316.1	78	22	5.70	1.00	1000	KetoconazoleE
29	Atenolol	Sigma Aldrich	267.2–145.0	78	35	4.11	5.00	5000	Ketoconazole
30	Bisoprolol	Fluka	326.5–116.2	96	25	6.22	1.00	1000	Tiapride
31	Carvedilol	Sigma Aldrich	407.2–224.0	90	31	6.52	1.00	1000	Ketoconazole
32	Esmolol	Sigma Aldrich	296.1–145.1	78	38	5.80	1.00	1000	Ketoconazole
33	Labetolol	NIFDC	329.2–162.0	78	35	6.04	5.00	5000	Ketoconazole
34	Metoprolol	Sigma Aldrich	268.1–133.0	90	37	5.63	5.00	5000	Ketoconazole
35	Propranolol	Sigma Aldrich	260.0–116.0	90	24	6.38	5.00	5000	Ketoconazole
36	Sotalol	Sigma Aldrich	273.1–133.0	70	36	3.86	5.00	5000	Tiapride
α-adrenoceptor antagonists (α-blockers)									
37	Alfuzosin	Sigma Aldrich	390.2–235.1	114	38	5.82	1.00	1000	Ketoconazole
38	Doxazosin	Sigma Aldrich	452.1–344.1	126	39	6.60	5.00	5000	Tiapride
39	Methyldopa	USP	212.0–166.0	75	21	3.16	50.0	50,000	Tiapride
40	Phentolamine	Sigma Aldrich	282.1–212.1	55	25	6.10	1.00	1000	Tiapride

(*continued on next page*)

TABLE 3. (*Continued*) Fifty-five Antihypertensive Chemical Entities and Mass Spectrometry Parameters

No.	Antihypertensive Agents	Standard Manufacturer	Q1–Q3 (Da)	Declustering Potential (V)	Collision Energy (eV)	t_R, min	LLOQ, ng/mL	ULOQ, ng/mL	Internal Standard
41	Prazosin	Fluka	384.1–247.0	116	39	5.88	1.00	1000	Ketoconazole
42	Terazosin	USP	388.2–290.1	84	37	5.67	5.00	5000	Diclofenac
43	Urapidil	NIFDC	388.2–233.1	92	47	5.40	20.0	20,000	Ketoconazole
Diuretics									
44	Amiloride	NIFDC	230.1–170.9	69	24	4.58	5.00	5000	Ketoconazole
45	Bendroflumethiazide	Fluka	420.0–289.0	−113	−32	5.60	1.00	1000	Glipizide
46	Bumetanide	NIFDC	365.1–240.0	90	25	8.28	5.00	5000	Ketoconazole
47*	Canrenone (Spironolactone Meta)	NA	341.1–107.1	87	38	8.61	50.0	50,000	Dexamethasone
48	Chlorthalidone	Fluka	337.0–145.9	−78	−29	4.96	1.00	1000	Glipizide
49	Furosemide	Fluka	328.8–285.0	−59	−21	5.55	20.0	20,000	NA (External Standard)
50	Hydrochlorothiazide	Sigma Aldrich	296.0–269.0	−80	−26	4.10	5.00	5000	Glipizide
51	Indapamide	Fluka	366.0–132.0	78	20	7.18	5.00	5000	Dexamethasone
52	Torasemide	NIFDC	349.1–264.1	63	22	6.92	1.00	1000	Tiapride
Others									
53	Aliskiren	Adoog Bioscience	552.4–436.1	81	26	7.41	1.00	1000	Ketoconazole
54	Hydralazine	Sigma Aldrich	161.1–89.0	73	29	3.49	50.0	50,000	Tiapride
55	Moxonidine	Sigma Aldrich	242.2–198.9	100	32	4.24	5.00	5000	Ketoconazole

*The original formats of these drugs were not able to detect because of its fast metabolism, but the stable metabolites of these drugs in urine can be detected by our method to confirm if the drugs were taken. These drugs were not quantified because of the lack of their metabolite standard availabilities; thus, qualitative results are only reported.

Meta, metabolite; t_R, retention time; ULOQ, upper limit of quantification.

tubes were vortexed for 2 minutes again then centrifuged at 14,000 rpm for 5 minutes.

Assessments of Specificity, Standard Curve, Accuracy, Precision, and Sample Stability

The specificity of the method was evaluated by extracting blank human urine and blank plus internal standard samples to determine the selectivity of the method and confirming whether or not the interference peak was observed with the lower limit of quantification (LLOQ) in the standard curve.

The standard curve included at least 8 nonzero points, one double blank, and one zero point (single blank). One set of standard curves was placed at the beginning of the analysis lot, and the other set was placed at the end of the analysis lot. The linear range of the analytical method was obtained by linear regression using a weighted least squares method (weighting factor L/conc2) for the standard curve of each drug (2 parallel samples per sample).

Accuracy and precision of the method were obtained by analyzing human urine control samples distributed at low, medium, and high concentrations in the linear range. Precision was represented by the coefficient of variation. The accuracy was measured by the deviation between the measured value and the theoretical value calculated by the following formula (Bias %): Bias (%) = $[(C_m - C_n)/C_n] \times 100\%$ where C_m is the average of all measured values at each concentration level and C_n is the theoretical value for each concentration level. Intraday accuracy and precision were calculated by analyzing 6 parallel samples of each control sample concentration level. Interday accuracy and precision were calculated by analyzing 18 parallel samples of each of the 3 control batch concentration points.

The long-term freeze stability of urine samples stored at −20°C (+5/−10°C) was evaluated by measuring QC samples at low, medium, and high levels. Urine samples were stored for 27 days at −20°C (+5/−10°C) and analyzed with the newly prepared standard curve. Stability was evaluated from the measured values.

Method Validation: a Pilot Study in 21 Hospitalized Patients

To further validate whether our established LC-MS/MS method reported in this study was able to detect antihypertensive drugs in daily clinical practice. Urine samples from 21 hypertensive in-hospital patients were collected 40 minutes after orally taking antihypertensive drugs and used for examining what drugs these patients have taken. The drugs that each patient took were not known by the testers, and the test results were confirmed against the prescriptions for the patients.

The clinical protocol described as above was approved by the Ethics Committee of the First Affiliated Hospital with Nanjing Medical University (Approval Number: 2017-SR-238), Nanjing, China.

Utilization of the LC-MS/MS Method to Ensure Drug Compliance in a Catheter-based Trial to Treat Hypertension

During this trial, to exclude interferences of antihypertensive drugs as a confounder, enrolled subjects followed a rigorous drug regimen and 5 antihypertensive drugs were allowed during the trial: irbesartan, metoprolol, amlodipine, hydrochlorothiazide, and terazosin. Urine samples were collected from 92 consecutive subjects after they completed a 28-day run-in phase, at month 3 and month 6 during a 6-month follow-up period and tested by our LC-MS/MS method to explore if these patients had followed the regimen or took any other antihypertensive medications outside of what was prescribed. These subjects were closely managed by a fully dedicated team to remind the subjects about their drug regimen through phone calls, text messages, and out-patient visits at the end of the run-in phase and at the month 1, 2, 3, 4, 5 and month 6 follow-ups. The results of LC-MS/MS were cross-referenced and confirmed by physician prescriptions, patient diaries, and tablet counts.

This clinical protocol was approved by the Ethics Committee of the First Affiliated Hospital with Nanjing Medical University (Approval Number: 2015-MD-228) and registered at www.clinicaltrials.gov, identifier NCT02761811.

RESULTS

Specificity

The results showed that under the selected conditions, the endogenous compounds in the urine sample had no obvious interference with the corresponding channels of the samples; the peak shape of the sample was as predicted, and there was no impurity peak interference. The chromatographic detection retention time of each chemical entity is shown in Table 3. The established LC-MS/MS method had excellent selectivity, and representative chromatogram tracings are shown in Figure 1.

Standard Curve

Considering that 5 antihypertensive drugs in our library are rapidly metabolized in the human blood circulation system, the drug metabolites were detected to determine whether the antihypertensive drugs were taken. Therefore, each of the hypertensive drugs and 5 of their corresponding metabolites had linear ranges within the selected concentration range. The concentration range was selected as the range of urine excretion concentration corresponding to the PK drug metabolism curve. The average return concentration of each concentration level was within ±15% of the indicated value, and LLOQ does not exceed ±20%. The correlation coefficient γ of each compound detection standard curve was greater than 0.99. The linear range of 27 antihypertensive drugs was 1.00–1000 ng/mL, 18 drugs was 5.00–5000 ng/mL, 5 drugs was 20.0–20,000 ng/mL, and another 5 drugs was 50.0–50000 ng/mL, which are summarized in Table 3.

Accuracy and Precision

Intraday and interday accuracy and precision were tested by analyzing 6 parallel samples of each QC level sample concentration with 3 levels of QCs; a total of 18 QC samples were analyzed. The accuracy and precision for each QC level were determined using the 6 samples at their respective QC level. The relative standard deviations (RSD values) of the intraday and interday as defining the precision of the low, medium, and high concentrations of the method were all less than 15.0%. The accuracies from each level are higher than 85%. The accuracy and precision in this method achieved the levels as we designed.

Stability

The long-term freeze stabilities of urine samples, stored at −20°C for 27 days, were evaluated by measuring QC samples at 3 levels of low, medium, and high concentrations. The QC samples were stored as the same conditions with the urine samples, which were stored at −20°C. At day 27, the QCs were analyzed together with the freshly prepared standard curve. Stabilities were evaluated from the measured values. The results showed that the various antihypertensive drugs (except hydralazine, flunarizine, lercanidipine, fosinopril, and lacidipine) were stable for at least 27 days at −20°C.

Method Validation: a Pilot Study in 21 Hospitalized Patients

The results from this pilot study to validate our LC-MS/MS method demonstrated that 5 categories of antihypertensive drugs were taken by 21 hospitalized patients: angiotensin-converting enzyme inhibitor, angiotensin II receptor inhibitors, beta-blockers, calcium channel blockers, alpha-blockers, and diuretics. Drugs detected by our LC-MS/MS method are listed in Table 4. The results were identical to the prescriptions by physicians because these patients were closely managed during their hospitalizations.

Utilization of the LC-MS/MS Method to Ensure Drug Compliance in a Clinical Study

Ninety-two consecutive patients were enrolled in the study, and the compliances of these patients during the run-in period, at 3 month and the 6-month follow-up are shown in Table 5. The results revealed that antihypertensive drugs detected in urine samples from 85 subjects were consistent with their prescriptions during the 28-day run-in periods. However, 7 subjects were detected by LC-MS/MS for their noncomplianceat the 28th day, 1 was detected at 3 month and 3 were detected at 6 month (Table 6). The names and concentrations of these drugs are listed in Table 6, and the chromatograms of LC-MS/MS from 1 of these 11 patients are shown in Figure 2. Among these 11 patients, antihypertensive drugs outside of their prescriptions were found in 7 of these patients' urine samples. We carefully conducted inquiries on these patients, examined their diary and drug counts, and found that these 7 subjects indeed took antihypertensive drugs outside of their prescriptions. One subject forgot to take the prescribed medication, and thus, we were unable to find antihypertensive drugs in his urine samples.Three patients did not take one of prescribed drugs for them. Per results of LC-MS/MS, we corrected the errors of the patients and ensured drug compliance during the trial.

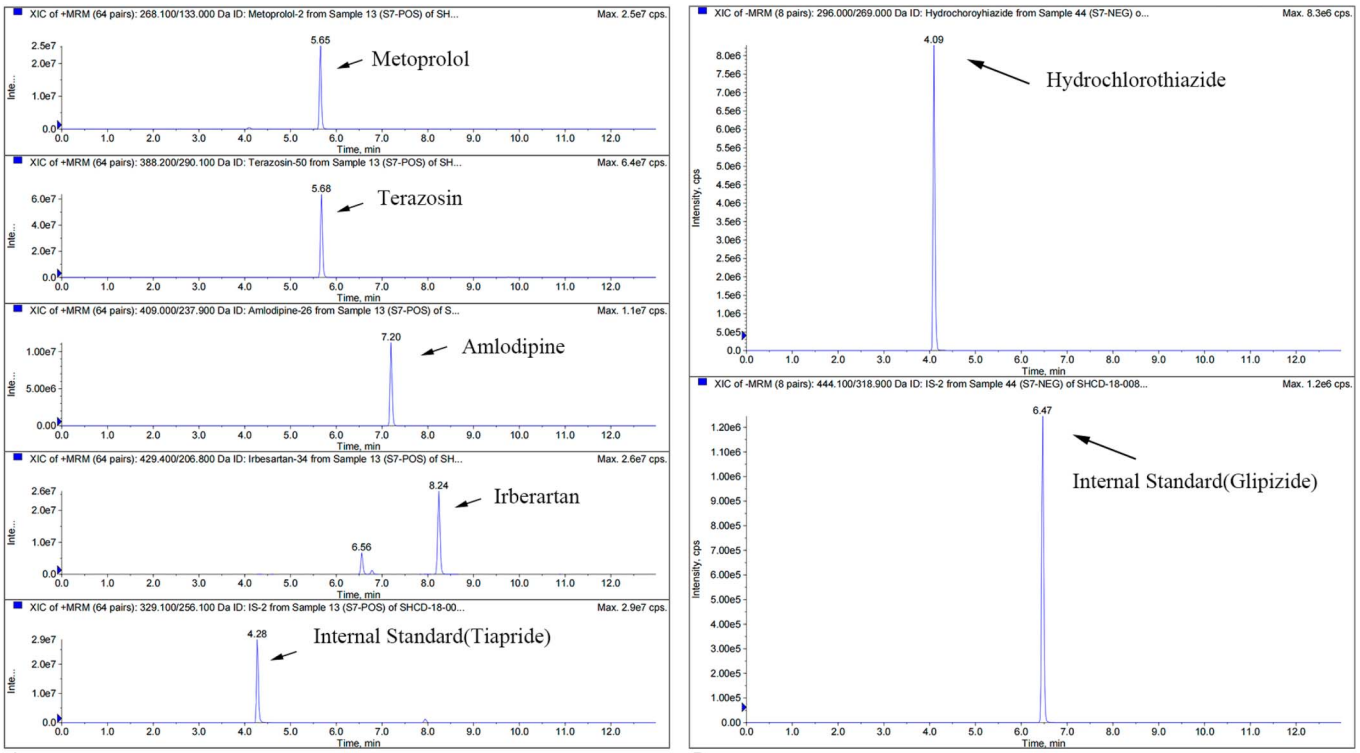

FIGURE 1. Representative chromatograms of antihypertensive drugs. A, Positive ion detection. The representative mass spec chromatograms of positive ion detection for metoprolol, terazosin, amlodipine, irbesartan, and an example of internal standard (tiapride) are shown; in this case, tiapride is used for the determination of irbesartan. B, Negative ion detection. The representative mass spectrometry chromatograms of negative ion quantitation for hydrochlorothiazide and its internal standard, glipizide, are shown.

Subsequently, drug compliance was maintained during the 3-month (97.8%) and 6-month (95.5%) follow-up periods (Table 5).

DISCUSSION

The successes of both chronic disease management and clinical trials are heavily determined by drug adherence. Traditional approaches, including prescription tracking, drug tablet counting, patient self-report, and electronic medication monitoring systems, have been used to monitor drug adherence. However, the reliability of these methods is subjective to human errors. Here, we developed methods of LC-MS/MS to detect antihypertensive drugs in urine and used the methods to ensure drug compliance during a clinical trial of device-based therapy for treatment of hypertension.

This study is not the first but rather one of few to report on the very complicated environment of drug therapy management of hypertensive patients in China. There are at least 172 antihypertensive drugs used, which include 55 chemical entities. We did not realize that so many generic antihypertensive drugs are currently being used in China until this study. The reality of the situation may be more severe than what our study reveals because many NMPA-approved (the regulatory agency of central government, previous name was CFDA) traditional Chinese herbal drugs with antihypertensive labeling are also used by physicians in China. Many of these medications can be obtained by patients from pharmacies without a prescription. Thus, to manage proper drug compliance for patients with hypertension or for a clinical trial of an antihypertensive therapy, the challenge is particularly severe. In addition, the poor drug compliance habit among Chinese patients is not yet taken into consideration as an issue.[10] Furthermore, it has been accepted that conventional approaches are insufficient at monitoring drug compliance in general. The task of managing medication adherence is even more challenging in this environment.

LC-MS/MS is a gold standard to monitor patient compliance with multiple antihypertensive drugs using either urine or plasma samples.[19,20] Several groups of investigators reported their studies regarding the use of LC-MS/MS or similar techniques for monitoring compliance. Jung et al used LC-MS to detect 368 antihypertensive drugs in urine samples from patients with resistant hypertension and examined whether these patients were truly drug resistant or had compliance issues. They found that among patients who met the criteria of resistance hypertension 53% were nonadherent detected by LC-MS. However, their method failed to detect lercanidipine and nitrate drugs.[21] They also neither did report how the specificity, accuracy, and precision of their LC-MS were assessed nor validations of the methods. Using the LC-MS/MS method, Tomaszewski et al revealed high rates of nonadherence to drug therapy among hypertensive patients: 25% of 208 hypertensive patients were nonadherent to their

TABLE 4. Antihypertensive Drugs Detected by LC-MS/MS in a Pilot Study (n = 21)

Patient No.	Prescription	Tested Drugs, ng/mL	If Consistent With Prescription?
1	None	None	Yes
2	Losartan potassium	Losartan (1596.5 ng/mL)	Yes
3	Amlodipine Besylate	Amlodipine (468.1 ng/mL)	Yes
	Olmesartan	Olmesartan (145.8 ng/mL)	
	Valsartan	Valsartan (16,825.3 ng/mL)	
4	Telmisartan	Telmisartan (42.7 ng/mL)	Yes
5	Candesartan cilexetil	Candesartan (116.3 ng/mL)	Yes
6	None	None	Yes
7	Furosemide	Furosemide (781.5 ng/mL)	Yes
	metoprolol tartrate	Metoprolol (312.6 ng/mL)	
8	Amlodipine besylate	Amlodipine (247.2 ng/mL)	Yes
	Irbesartan	Irbesartan (292.0 ng/mL)	
	metoprolol tartrate	Metoprolol (1862.4 ng/mL)	
9	Irbesartan	Irbesartan (179.0 ng/mL)	Yes
	metoprolol tartrate	Metoprolol (16.2 ng/mL)	
10	Amlodipine besylate	Amlodipine (127.9 ng/mL)	Yes
	Metoprolol succinate	Metoprolol (298.8 ng/mL)	
11	Metoprolol tartrate	Metoprolol (1669.1 ng/mL)	Yes
12	Furosemide	Furosemide (4744.0 ng/mL)	Yes
	Metoprolol tartrate	Metoprolol (530.0 ng/mL)	
	Spironolactone	Spironolactone Meta (1363.0 ng/mL)	
13	Amlodipine Besylate	Amlodipine (187.2 ng/mL)	Yes
	Losartan potassium	Losartan (713.4 ng/mL)	
	Metoprolol Succinate	Metoprolol (453.3 ng/mL)	
14	Amlodipine Besylate	Amlodipine (545.0 ng/mL)	Yes
	Irbesartan	Irbesartan (263.8 ng/mL)	
	Spironolactone	Spironolactone Meta (245.6 ng/mL)	
15	Amlodipine Besylate	Amlodipine (734.6 ng/mL)	Yes
	Diltiazem	Diltiazem (3038.69 ng/mL)	
	Metoprolol Succinate	Metoprolol (1432.7 ng/mL)	
	valsartan	Valsartan (2264.1 ng/mL)	
16	Hydrochlorothiazide	Hydrochlorothiazide (1423.3 ng/mL)	Yes
	Irbesartan	Irbesartan (559.1 ng/mL)	
	Metoprolol tartrate	Metoprolol (799.3 ng/mL)	
17	Hydrochlorothiazide	Hydrochlorothiazide (405.0 ng/mL)	Yes
	Irbesartan	Irbesartan (114.3 ng/mL)	
18	Terazosin	Terazosin (47.0 ng/mL)	Yes
19	Felodipine	Felodipine Meta (927.4 ng/mL)	Yes
	Terazosin	Terazosin (37.4 ng/mL)	
20	Hydrochloride Terazosin	Terazosin (65.7 ng/mL)	Yes
21	Hydrochlorothiazide	Hydrochlorothiazide (3498.5 ng/mL)	Yes
	Irbesartan	Irbesartan (132.0 ng/mL)	
	Spironolactone	Spironolactone Meta (1945.3 ng/mL)	

antihypertensive drugs. Among the 25%, total nonadherence was 10.1% and partial nonadherence was 14.9%. Their methods were able to monitor 40 antihypertensive drugs in urine.[22] To monitor adherence in patients with hypertension, a group of Italian investigators established methods of UHPLC-MS/MS to detect 10 antihypertensive drugs in plasma and urine.[23,24] The investigators monitored the concentrations of these 10 drugs in plasma samples from 22 hypertensive patients, and results showed a good correlation between drug concentrations and prescriptions.

Our LC-MS/MS method was able to detect 172 antihypertensive drugs and covered 55 chemical entities including a broad spectrum of drugs that treat hypertension: angiotensin-converting enzyme inhibitors, angiotensin II receptor blocker, calcium channel blockers, β-adrenoceptor blockers, α-adrenoceptor blockers, diuretics, vasodilators/nitrates, and compound drugs. To the best of our knowledge, the antihypertensive drugs detected by our own methods are much broader compared with other hypertension trials. Our methods were not only able to detect drugs within the studied therapeutic regimens but also

TABLE 5. Antihypertensive Drug Adherence Monitored by LC-MS/MS During a Device-based Clinical Trial for Treatment of Hypertension (n = 92)

If Consistent With Prescription? (Subjects)	Baseline	3M Follow-Up	6M Follow-Up
Yes	85 (92.4%)	90 (97.8%)	86 (96.6%)
No	7 (7.6%)	1 (1.1%)	3 (3.4%)
Withdrew from the trial	0	1	3

detect drugs used in general clinical practice. The accuracy and precision of the methods were evaluated by bench testing and further by a pilot study using clinical urine samples, showing that drugs in urine samples matched with the prescriptions of these patients. The stability of these drugs or its metabolites in urine samples were in a well acceptable range (−20°C for 27 days) because the urine samples were shipped to the central laboratory within 2 days after the samples were taken. Thus, this is a power tool to ensure drug adherence of our device-based therapy to treat hypertension meanwhile excluding the interference of drugs outside of what have been prescribed. Using the methods, we had indeed sufficiently managed drug compliance of hypertensive patients who were enrolled in the device-based therapy study and maintained their adherence to prescriptions at a significantly high level.

It has been well accepted that drug compliance is a crucial confounder to evaluate the efficacy of RDN therapy for treatment of hypertension.[25,26] Thus, to reliably monitor and manage drug compliance becomes a determining factor for RDN trials.[27] LC-MS/MS techniques have been used to detect antihypertensive drugs in blood samples and to ensure patient's adherence by 2 well-known RDN trials: Spyral Global Off-Med and On-Med studies, although the investigators have not published data regarding results of LC-MS/MS assessment.[28] LC-MS/MS apparently becomes the gold standard for drug compliance in RDN trials.

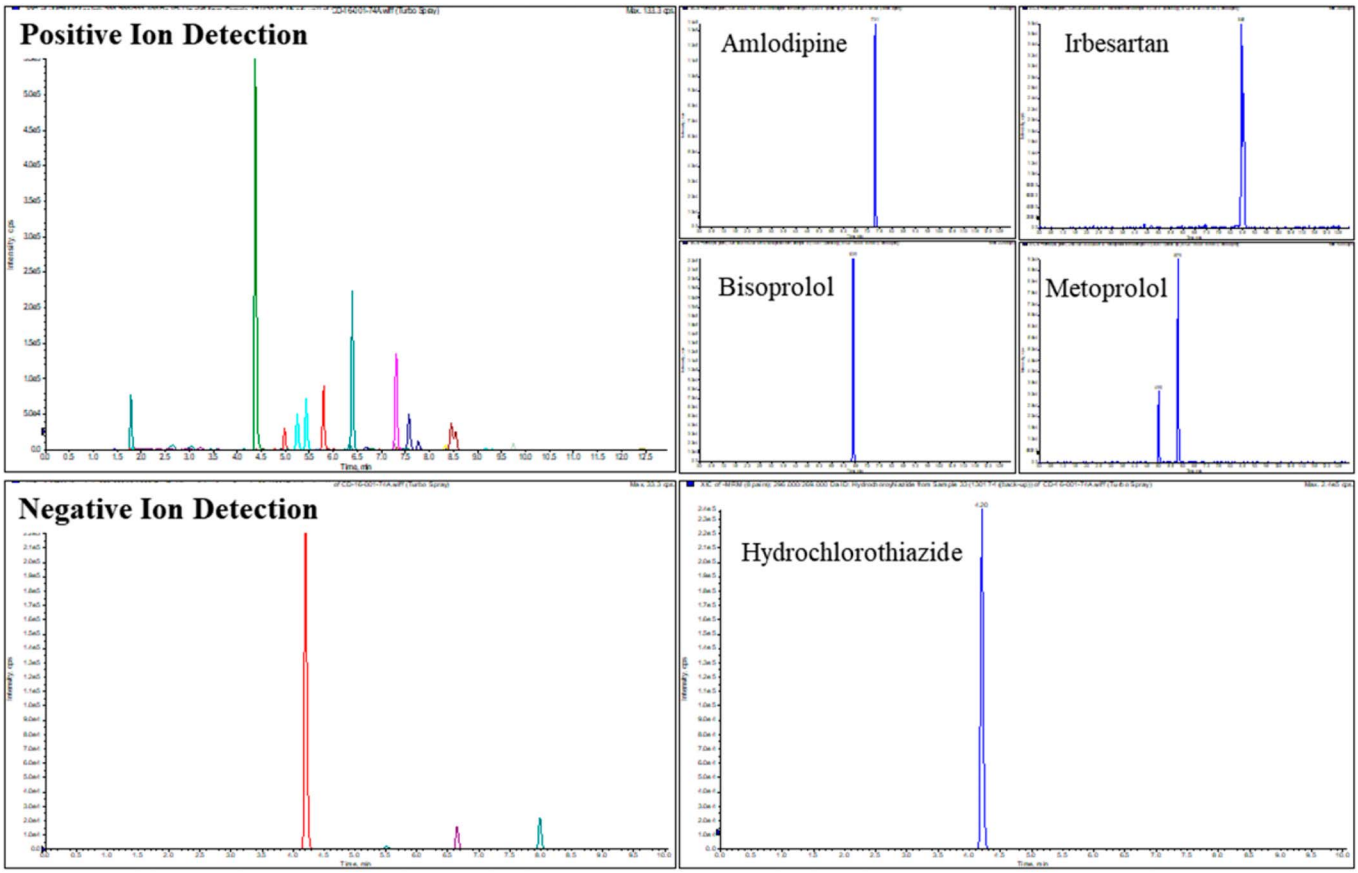

FIGURE 2. Representative chromatograms of antihypertensive drugs in urine sample from a patient. Amlodipine, metoprolol, irbesartan, hydrochlorothiazide, and bisoprolol were detected in the urine sample from No. 7 patient, in Table 6, bisoprolol was not prescribed for this patient.

TABLE 6. Antihypertensive Drug Concentrations in Urine From 11 Patients With Noncompliance

No.	Time Point	Prescription	Detected Drugs/Urine Concentration
1	28 days	Aprovel (irbesartan tablets)	Irbesartan (43.40 ng/mL)
		Norvasc (amlodipine besylate tablets)	Amlodipine (74.40 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Metoprolol (1757.00 ng/mL)
			Felodipine Meta (321.00 ng/mL)
			Nifedipine Meta (335.00 ng/mL)
			Spironolactone Meta (358.00 ng/mL)
2	28 days	Aprovel (irbesartan tablets)	Irbesartan (214.90 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Metoprolol (447.04 ng/mL)
			Losartan (5347.13 ng/mL)
3	28 days	Aprovel (irbesartan tablets)	Metoprolol (562.24 ng/mL)
		Norvasc (amlodipine besylate tablets)	
		Betaloc SR (metoprolol succinate sustained-release tablets)	
		Hytrin (terazosin hydrochloride tablets)	
		Coaprove (irbesartan and hydrochlorothiazide tablets)	
4	28 days	Aprovel (irbesartan tablets)	Irbesartan (22.46 ng/mL)
		Norvasc (amlodipine besylate tablets)	Amlodipine (362.79 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Hydrochlorothiazide (1994.46 ng/mL)
		Changyao (hydrochlorothiazide tablets)	Nifedipine Meta (91.61 ng/mL)
5	28 days	Betaloc SR (metoprolol succinate sustained-release tablets)	Metoprolol (1120.9 ng/mL)
			Irbesartan (92.6 ng/mL)
		Coaprove (irbesartan and hydrochlorothiazide tablets)	Hydrochlorothiazide (3107.4 ng/mL)
			Amlodipine (93.0 ng/mL)
6	28 days	Aprovel (irbesartan tablets)	Irbesartan (115.01 ng/mL)
		Norvasc (amlodipine besylate tablets)	Amlodipine (246.50 ng/mL)
			Nifedipine Meta (6785.16 ng/mL)
7	28 days	Norvasc (amlodipine besylate tablets)	Amlodipine (1156.70 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Metoprolol (218.18 ng/mL)
			Irbesartan (159.63 ng/mL)
		Coaprove (irbesartan and hydrochlorothiazide tablets)	Hydrochlorothiazide (16,322.67 ng/mL)
			Bisoprolol (134.31 ng/mL)
8	3 months	Aprovel (irbesartan tablets)	Irbesartan (2238.00 ng/mL)
		Norvasc (amlodipine besylate tablets)	Amlodipine (62.24 ng/mL)
			Metoprolol (13.56 ng/mL)
9	6 months	Aprovel (irbesartan tablets)	Irbesartan (204.11 ng/mL)
		Norvasc (amlodipine besylate tablets)	Amlodipine (63.06 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	
10	6 months	Norvasc (amlodipine besylate tablets)	Amlodipine (648.44 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Metoprolol (136.76 ng/mL)
			Hydrochlorothiazide (2627.04 ng/mL)
		Coaprove (irbesartan and hydrochlorothiazide tablets)	
11	6 months	Norvasc (amlodipine besylate tablets)	Amlodipine (56.15 ng/mL)
		Betaloc SR (metoprolol succinate sustained-release tablets)	Irbesartan (539.16 ng/mL)
			Hydrochlorothiazide (3804.20 ng/mL)
		Coaprove (irbesartan and hydrochlorothiazide tablets)	

LC-MS/MS established in this study can be not only used for clinical trials but also used for daily clinical practice, in particular for diagnosing whether patients with uncontrolled blood pressure are true drug resistant or poor drug adherence,[8,21] it could allow physicians making rational therapeutic decisions.[21] However, the cost of LC-MS/MS needs to be taken into consideration, and this is an important limiting factor for large-scale application.[5]

Our method has several limitations. First, the methods can only qualitatively detect antihypertensive drugs and its metabolites in urine but are not able to evaluate the actual doses of the drugs taken. Second, once patients were administrated nitrate drugs simultaneously such as sodium nitroprusside, nitroglycerin, isosorbide dinitrate, and isosorbide, our methods are not able to distinguish these drugs. Third, Chinese herbal medications are widely used in China for treatment of hypertension. It has been reported that some of these herbal drugs may contain chemical entities that are the same as what is used in Western antihypertensive drugs. We did not investigate how many Chinese herbal medications are used in the surveyed hospitals, so our LC-MS/MS method has not been adjusted to identify these Chinese herbal drugs. Finally, our assessments of antihypertensive drug adherence based on results of LC-MS/MS could be overestimated because of bias caused by the phenomenon so-called "toothbrush effect"[29]: Some patients may improve their compliance of described drugs just before attending follow-up sessions. This phenomenon has been reported by investigators, indeed has impacts on patient's behaviors, and may change results of a clinical study.[30]

CONCLUSIONS

In this study, we reported that there were at least 172 antihypertensive drugs used in daily clinical practice in China. These drugs present 55 chemical compounds and 6 classes of antihypertensive drugs. The method of LC-MS/MS with specificity, accuracy, and precision was successfully developed to detect these 55 chemical entities in human urine. Using the method for a device-based clinical trial to treat hypertension, we managed medication adherent of patients to their drug regimen, maintained significant high level of compliance, and excluded the key confounder of drug interferences during the trial.

REFERENCES

1. Breckenridge A, Aronson JK, Blaschke TF, et al. Poor Medication adherence in clinical trials: consequences and solutions. *Nat Rev Drug Discov.* 2017;16:149–150.
2. World Health Organization. *Adherence to Long-Term Therapies: Evidence for Action*. Geneva, Switzerland: World Health Organization; 2003.
3. Vrijens B, Antoniou S, Burnier M, et al. Current situation of medication adherence in hypertension. *Front Pharmacol.* 2017;8:100.
4. Vrijens B, Vincze G, Kristanto P, et al. Adherence to prescribed antihypertensive drug treatments: longitudinal study of electronically compiled dosing histories. *Br Med J*. 2008;336:1114–1117.
5. Burnier M, Wuerzner G, Struijker-Boudier H, et al. Measuring, analyzing, and managing drug adherence in resistant hypertension. *Hypertension*. 2013;62:218–225.
6. Faselis C, Doumas M, Papademetriou V. Common secondary causes of resistant hypertension and rational for treatment. *Int J Hypertens*. 2011; 2011:236239.
7. Hyman DJ, Pavlik V. Medication adherence and resistant hypertension. *J Hum Hypertens.* 2014;29:213–218.
8. Durand H, Hayes P, Harhen B, et al. Medication adherence for resistant hypertension: assessing theoretical predictors of adherence using direct and indirect adherence measures. *Br J Health Psych*. 2018;23:949–966.
9. Wang Z, Chen Z, Zhang L, et al Status of hypertension in China: results from the China hypertension survey, 2012–2015. *Circulation.* 2018;137:2344–2356.
10. Lu J, Lu Y, Wang X, et al. Prevalence, awareness, treatment, and control of hypertension in China: data from 1.7 million adults in a population-based screening study (China PEACE Million Persons Prject). *Lancet*. 2017;390:2549–2558.
11. Jin Y, Jocobs L, Baelen M, et al Rationale and design of the investigator-steered project on intravascular renal denervation for management of drug-resistant hypertension (INSPiRED) trial. *J Am Soc Hypertens.* 2014;23:138–146.
12. Osterberg L, Blaschke T. Adherence to medication. *New Engl J Med.* 2005;353:487–497.
13. Cramer J, Rosenheck R, Kirk G, et al Medication compliance feedback and monitoring in a clinical trial: predictors and outcomes. *Value Health*. 2003;6:566–573.
14. Marrazzo JM, Ramjee G, Richardson BA, et al Tenofovir-based preexposure prophylaxis for HIV infection among African women. *New Engl J Med.* 2015;372:509–518.
15. Fudim M, Sobotka AA, Yin YH, et al. Selective vs. global renal denervation: a case for less is more. *Curr Hypertens Rep.* 2018;20:37.
16. Thukkani AK, Bhatt DL. Renal denervation therapy for hypertension. *Circulation*. 2013;128:2251–2254.
17. Onzenoort HA, Verberk WJ, Kroon AA, et al. Electronic monitoring of adherence, treatment of hypertension, and blood pressure control. *Am J Hypertens.* 2012;25:54–59.
18. Yan ZP, Chen YW. Comparative analysis of approved generic drug in China and America. *Chin New Drug*. 2007;16:1731–1733.
19. Pucci M, Martin U. Detecting non-adherence by urine analysis in patients with uncontrolled hypertension: rates, reasons and reactions. *J Hum Hypertens.* 2017;31:253–257.
20. Chytil L, Strauch B, Cvacka J, et al. Determination of doxazosin and verapamil in human serum by fast LC-MS/MS: application to document non-compliance of patients. *J Chromatogr B*. 2010;878:3167–3173.
21. Jung O, Gechter JL, Wunder C, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. *J Hypertens.* 2013;31:766–774.
22. Tomaszewski M, White C, Patel P, et al. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. *Heart*. 2014;100:855–861.
23. Nicolo AD, Avataneoa V, Rabbiab F, et al. UHPLC-MS/MS method with protein precipitation extraction for the simultaneous quantification of ten antihypertensive drugs in human plasma from resistant hypertensive patients. *J Pharm Biomed Anal.* 2016;129:535–541.
24. Nicolo A, Avataneo V, Rabbia F, et al. UHPLC-MS/MS method with sample dilution to test therapeutic adherence through quantification of ten antihypertensive drugs in urine samples. *J Pharm Biomed Anal.* 2017; 142:279–285.
25. de Jager RL, de Beus E, Beeftink MM, et al Impact of medication adherence on the effect of renal denervation: the SYMPATHY Trial. *Hypertension*. 2017;69:678–684.
26. Bhatt DL, Kandzari DE, O'Neill WW, et al A controlled trial of renal denervation for resistant hypertension. *N Engl J Med.* 2014;370:1393–1402.
27. Patel P, Gupta PK, White CMJ, et al. Screening for non-adherence to antihypertensive treatment as a part of the diagnostic pathway to renal denervation. *J Hum Hypertens.* 2016;30:368–373.
28. Tanna S, Ogwu J, Lawson G. Hyphenated mass spectrometry techniques for assessing medication adherence: advantages, challenges, clinical applications and future perspectives. *Clin Chem Lab Med.* 2020;58: 643–663.
29. Chtterjee JS. From compliance to concordance in diabetes. *J Med Ethics*. 2006;32:507–510.
30. Alcouffe F. Improvement of oral hygiene habits: a psychological approach 2-year data. *J Clin Peridontol*. 1988;15:617–620.