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Development of a LC–MS/MS method for simultaneous analysis of 20 antihistamines in dietary supplements

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LC-MS/MS를 이용한 건강기능식품 내 항히스타민 20종 동시분석법 개발

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Abstract: Recently, the consumption of dietary supplements has increased because of people's greater interest in health. Unfortunately, the sales of dietary supplements containing unauthorized substances such as drugs have also increased. We developed a rapid, accurate method for the simultaneous determination of antihistamines using liquid chromatography tandem mass spectrometry with a multiple reaction monitoring mode. The limit of detection (LOD) and limit of quantification (LOQ) of the instrument used in this method were in the ranges 0.0003-0.3 and 0.0009-0.6 μg mL^{-1}, respectively. The linearity of the method was > 0.99. The precision levels of the method were 0.1-9.8% (intra-day) and 0.3-9.6% (inter-day), and the levels of accuracy of the method were 82.7-115.0% (intra-day) and 84.3-113.0% (inter-day). The mean of recovery of the method was in the range of 83.9-117.9% and the RSD of the stability was less than 5.9%.

Key words: LC–MS/MS, antihistamines, dietary supplements, unauthorized substances, multiple reaction monitoring

1. Introduction

Histamine, a low-molecular-weight amine, is a natural body constituent. Histamine is synthesized from l-histidine by histidine decarboxylase and involved in neurotransmission, allergic inflammation, and immune

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modulation. Histamine exerts its effects through the interaction with one of the four distinct receptors (H_1, H_2, H_3, and H_4). The manifestation of receptors is changed depending on the level of cell differentiation and microenvironmental influences.[1] Histamine exhibits proinflammatory activity and is relevant to the development of diverse aspects of antigen-specific immune response by the H_1 receptor.[2-3,5] H_1 antihistamines are used in the treatment of allergic disorder, allergic rhinoconjunctivitis, and urticaria.[4,6] H_1 antihistamines directly inhibit the action of histamine through H_1 receptors on antiallergic inflammation in blood vessels and nerve cells. Moreover, antiallergic activities such as the anti-inflammatory functions of mast cells and basophils probably exert a direct inhibitory effect on calcium ion channel and NF-kB.[5,7] H_1 antihistamines are classified into alkylamines, piperazines, piperidines, ethanolamines, ethylenediamines, and phenothiazines according to the chemical structures or functional groups, which is determined by the permeability of the blood–brain barrier for classification as the first- or second-generation histamines.[5] There are large differences between the two H_1 antihistamine generations in terms of their propensity to cause the side effects of central nervous system (CNS). The first-generation H_1 antihistamines penetrate well into the CNS where they induce sedation. In contrast, the second-generation H_1 antihistamines allow much reduced CNS sedation.[6,8] According to the United States Food and Drug Administration-approved product labelling, potential side effects associated with first-generation antihistamines include cardiotoxicity, drowsiness, and cognitive impairment.[24]

Because of the growing interest in health and nutrition, the sale of dietary supplements steadily increases every year. Unfortunately, the adulteration of food ingredients and dietary supplements with various types of drugs is a concern, risking human health in long term. Liang *et al*.[23] reported that promethazine as adulterant was found in herbal medicine and food supplements, these products were declared having the effect of tranquilization or improving 'health status' of old people. The consumption of these adulterated products which contain chemical drugs could be harmful to consumers with various health risks. For this reason, more accurate and effective method is needed for rapid determination of adulteration such as antihistamines in foods or dietary supplements.

Previously, the simultaneous analysis of antihistamines using various instruments has been reported. HPLC has been used to analyze antihistamines by Arayne *et al*.[9] and Karakus *et al*.[10] Gergov *et al*.[11] simultaneously determined the concentration of 18 antihistamines in blood by liquid chromatography-ionspray tandem mass spectrometry (LC–MS/MS) with multiple reaction monitoring (MRM). Hasegawa *et al*.[12] simultaneously determined the concentration of antihistamines such as diphenhydramine, chlorpheniramine, triprolidine, promethazine, cyproheptadine, and clemastine by gas chromatography coupled with mass spectrometry (GC–MS). Moreover, levocetirizine and its isomers in human blood have been identified by LC–MS/MS. Thus, most of the simultaneous determinations of the concentrations of antihistamines have been conducted only in the blood or urine samples from human, not in adulterated food or dietary supplements, and by liquid chromatography coupled with UV detection (LC–UV) or LC–MS.[13-21]

In this paper, we report a rapid and accurate method for the simultaneous determination of the concentrations of antihistamines in various types of dietary supplements that were intentionally adulterated with antihistamines by LC–MS/MS.

2. Materials and Methods

2.1. Reagents and chemicals

Acrivastine (ACV), astemizole (AMZ), azelastine hydrochloride (AZT), brompheniramine maleate (BPA), cetirizine (CTZ), chlorpheniramine maleate (CPA), clemastine fumarate salt (CMT), cyproheptadine hydrochloride sesquihydrate (CHD), desloratadine (DLD), diphenhydramine hydrochloride (DPH), ebastine (EBT), epinastine hydrochloride (EPT), fexofenadine hydrochloride (FFD), hydroxyzine hydrochloride (HXZ), ketotifen fumarate salt (KTF), loratadine (LTD), olopatadine hydrochloride (OPD),

promethazine hydrochloride (PMZ), terfenadine (TFD), and triprolidine hydrochloride (TPD) were purchased from USP (Rockville, MD, USA) and Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI, USA). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). High-purity deionized water (DW) was prepared using Milli-Q (Millipore, Bedford, MA, USA) purification system. The standard stock solutions (1,000 μg/mL) were prepared by dissolving an appropriate amount of the drug in methanol. Each stock solution was stored at 4 °C. Standard working mixtures were prepared daily by diluting the stock solutions with methanol for each calibration curve and method validation.

2.2. Apparatus

A Waters Acquity UPLC system (Waters, Milford, MA, USA) equipped with binary pumps, a sample manager, and a column oven was used for the experiments. The liquid chromatographic separation was performed using an Acquity UPLC BEH C_{18} (1.7 μm, 2.1 × 100 mm, Waters, Milford, MA, USA) column with the temperature of the column set at 40 °C. The flow rate was 0.25 mL/min, and the injection volume was 2 μL. The mobile phase consisted of 0.1% formic acid in DW (A) and 0.1% formic acid in acetonitrile (B). The gradient condition of the mobile phase was as follows: 0 min, 5% B; 1.0 min, 5% B; 7.0 min, 100% B; 8.0 min, 100% B; 8.1 min, 5% B; and 10.0 min, 5% B. An MS system manufactured by Waters Xevo TQMS (Waters, Milford, MA, USA) was operated using MRM with electrospray ionization (ESI) in positive ion mode. The desolvation temperature was 400 °C, and the flow rates of the desolvation and cone gases were set at 600 L/h and 40 V, respectively. For optimizing the analytical conditions, the MS capillary voltage was set at 2.7 kV.

2.3. Sample preparation

We purchased different types of dietary supplements such as powders, capsules, tablets, liquids, and pills from the South Korean markets for method validation.

Table 1. MRM[a)] transitions for 20 antihistamine compounds

Compound	Ion mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Cone voltage (kV)
ACV	ESI (+)	349.29	232.15, 260.17, 278.18	36, 26, 16	22
AMZ	ESI (+)	459.27	135.00, 218.07, 308.07	35, 25, 30	40
AZT	ESI (+)	382.29	112.13, 159.06, 271.20	24, 36, 30	34
BPA	ESI (+)	319.22	167.06, 245.20, 274.12	44, 38, 20	38
CTZ	ESI (+)	403.20	166.01, 200.97, 367.10	40, 20, 15	25
CPA	ESI (+)	275.13	167.06, 201.00, 230.05	35, 34, 10	20
CMT	ESI (+)	344.27	130.05, 180.02, 215.01	10, 33, 15	15
CHD	ESI (+)	288.29	191.12, 202.17, 215.17	32, 52, 40	34
DLD	ESI (+)	311.23	259.21, 282.20, 294.22	25, 20, 25	30
DPH	ESI (+)	256.22	152.06, 165.13, 167.08	36, 38, 18	14
EBT	ESI (+)	470.32	167.00, 203.08, 302.12	35, 25, 20	30
EPT	ESI (+)	250.16	130.99, 193.15, 208.17	34, 36, 28	44
FFD	ESI (+)	502.46	171.15, 466.47, 484.50	40, 30, 35	38
HXZ	ESI (+)	375.32	165.05, 166.06, 201.08	50, 40, 20	20
KTF	ESI (+)	310.23	96.02, 213.11	20, 30	35
LTD	ESI (+)	383.29	259.13, 267.06, 337.21	36, 36, 30	38
OPD	ESI (+)	338.29	141.06, 165.11, 247.16	28, 26, 24	34
PMZ	ESI (+)	285.22	86.05, 198.06, 240.17	16, 22, 20	20
TFD	ESI (+)	472.32	262.10, 436.20, 454.22	40, 30, 35	38
TPD	ESI (+)	278.29	167.07, 193.09, 208.17	16, 22, 20	20

[a)]Multiple monitoring reaction

Each sample was homogenized, and 1 g of the sample was placed in a 20 mL volumetric flask. Then, the sample was extracted with methanol by sonication for 10 min. Finally, the extract was filtered through a 0.22-μm PVDF filter (Millipore, Milford, MA, USA) before the injection for analysis.

3. Results

3.1. Optimization of LC-MS/MS conditions

The MS/MS optimization was performed by the direct infusion of each individual standard solution. Precursor ion discovery (PID) and LC-MS/MS scans were carried out using ESI operated in positive mode. The protonated molecular ion and two or three fragment ions were selected as the precursor and product ions, respectively. The instrument parameters such as collision energy, cone voltage, desolvation temperature, and capillary voltage were tuned to obtain the optimal intensity of product ion. The optimized MRM conditions are shown in *Table* 1. To optimize the separation of 20 antihistamine compounds, various compositions of mobile phase with different buffers such as ammonium acetate and formic acid were used.

The mobile phase without any buffer showed low peak intensity. Moreover, a broad peak shape and tailing were observed when ammonium acetate buffer was used, while the use of formic acid showed a good peak intensity and separation. When an Acquity UPLC BEH C_{18} column was used, the compounds could be effectively separated in terms of peak shape and intensity compared to those obtained using BEH C_8 (1.7 μm, 2.1 × 100 mm, Waters, Milford, MA, USA) and HSS T_3 column (1.8 μm, 2.1 × 100 mm, Waters, Milford, MA, USA). Therefore, the BEH C_{18} column was selected because it showed the best separation of the 20 antihistamine compounds. The LC–MS/MS chromatograms of the 20 antihistamine compounds are shown in *Fig.* 1.

3.2. Method validation

The developed LC–MS/MS method was validated according to the International Conference on Harmonization (ICH) guidelines.[22] The method was validated with regard to specificity, limit of detection

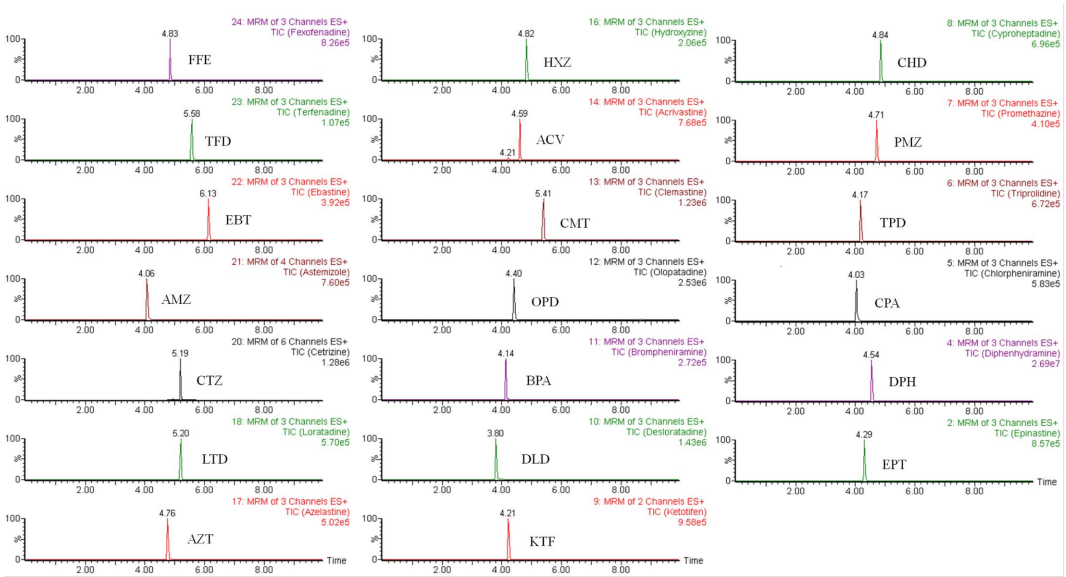

Fig. 1. Typical chromatogram of LC–MS/MS for 20 antihistamines: 1, fexofenadine (FFD); 2, terfenadine (TFD); 3, ebastine (EBT); 4, astemizole (AMZ); 5, cetirizine (CTZ); 6, loratadine (LTD); 7, azelastine (AZT); 8, hydroxyzine (HXZ); 9, acrivastine (ACV); 10, clemastine (CMT); 11, olopatadine (OPD); 12, brompheniramine (BPA); 13, desloratadine (DLD); 14, ketotifen (KTF); 15, cyproheptadine (CHD); 16, promethazine (PMZ); 17, triprolidine (TPD); 18, chlorpheniramine (CPA); 19, diphenhydramine (DPH); 20, epinastine (EPT).

(LOD), limit of quantification (LOQ), linearity, precision, accuracy, recovery and stability.

3.2.1. Specificity

The specificity of the method was evaluated by comparing the chromatograms obtained from the blank sample and spiked blank sample. The analysis of the 20 antihistamine compounds was carried out by injecting a blank sample (a pill type), and then the blank sample was spiked with the antihistamine

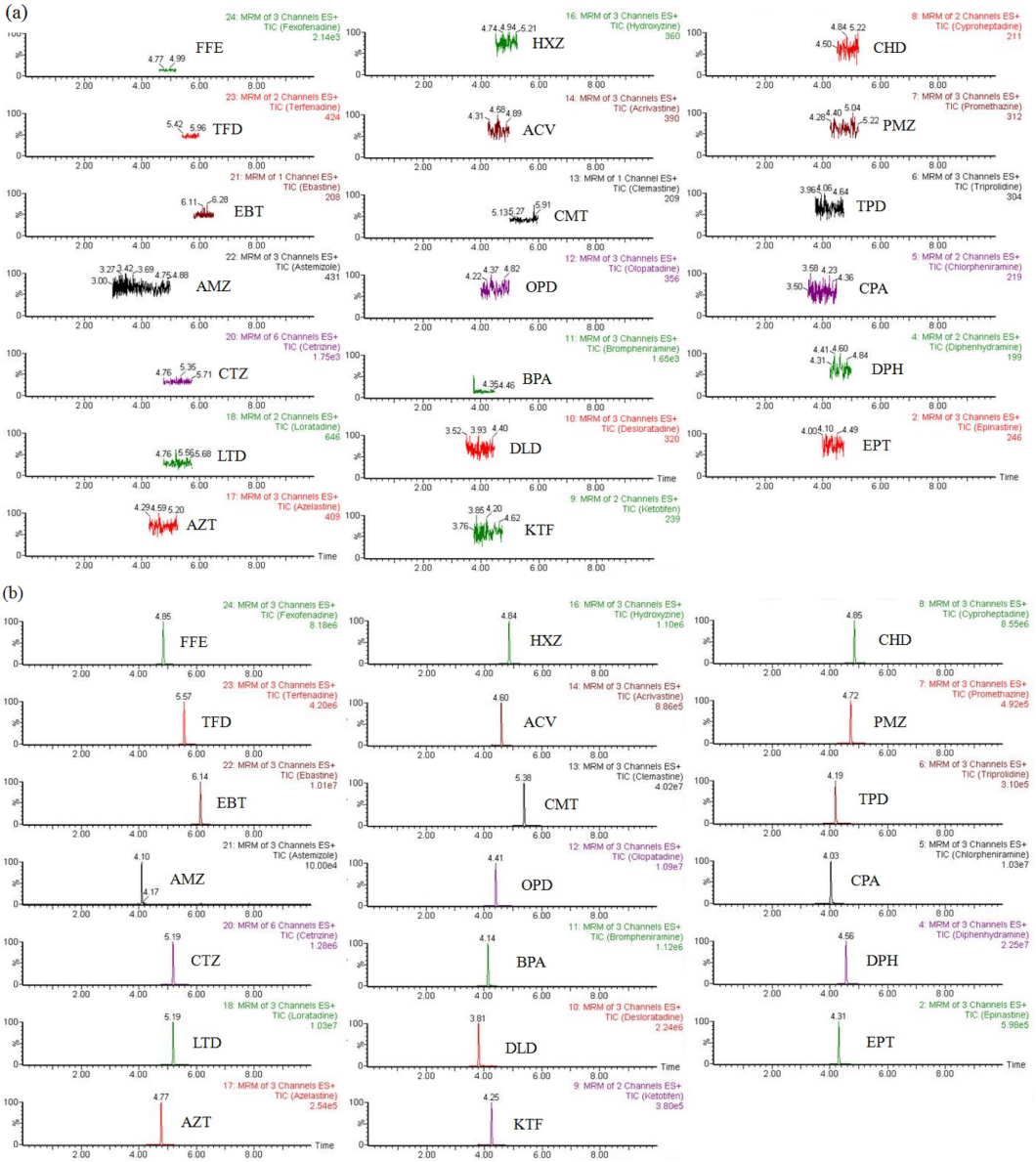

Fig. 2. MRM chromatograms of LC–MS/MS for 20 antihistamines: (a) blank sample, (b) spiked sample with antihistamines: Peak assignments: 1, fexofenadine (FFD); 2, terfenadine (TFD); 3, ebastine (EBT); 4, astemizole (AMZ); 5, cetirizine (CTZ); 6, loratadine (LTD); 7, azelastine (AZT); 8, hydroxyzine (HXZ); 9, acrivastine (ACV); 10, clemastine (CMT); 11, olopatadine (OPD); 12, brompheniramine (BPA); 13, desloratadine (DLD); 14, ketotifen (KTF); 15, cyproheptadine (CHD); 16, promethazine (PMZ); 17, triprolidine (TPD); 18, chlorpheniramine (CPA); 19, diphenhydramine (DPH); 20, epinastine (EPT).

compounds. The LC–MS/MS analysis of the 20 antihistamine compounds showed good sensitivity without interference from the sample matrix. *Fig.* 2 shows the chromatograms of the blank and spiked samples. The other forms of dietary supplements showed a similar trend, such as pills (data not shown).

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ) and linearity

The LOD and LOQ were determined from the standard deviation of the response of the injected target compounds prepared with a spiked blank sample or reference material (a pill type) at a signal-to-noise ratio of 3 and 10, respectively. The linearity of the compounds was in the concentration range 0.01-20 μg/mL. The determination of the LOD and LOQ were based on a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. The LOD and LOQ of the instrument used represent the LOD and LOQ concentrations of the compounds in the analyzed solutions, whereas the LOD and LOQ of the method represent the LOD/LOQ concentrations in the dietary supplements investigated. The LOD and LOQ of the instrument were determined as 0.0003-0.3 and 0.0009–0.6 μg/mL, respectively. The calibration curves of the 20 antihistamine compounds were constructed at six different concentration levels, and they showed excellent linear correlation (>0.99) (*Table* 2).

3.2.3. Accuracy and precision

The intra-day and inter-day assay precision were determined by repeated analysis in a single day (n = 3) and three different days (n = 3), respectively, and evaluated by the relative standard deviation (RSD). The intra-day and inter-day assay accuracy of the method was determined by adding a known amount of each compound corresponding to six independent concentration levels in triplicate. The intra-day and inter-day accuracy of the method calculated as the recovery. The RSD of the recovery was 0.3-9.6% (inter-day precision) and 0.1-9.8% (intra-day precision) for all the antihistamines (*Table* 3). The accuracy of the method was calculated by comparing the measured concentrations with the known spiked concentrations in triplicate. As shown in *Table* 4, the mean of recovery was 82.7-115.0% (intra-day accuracy) and

Table 2. Summary of the calibration curve, limit of detection (LOD), limit of quantification (LOQ)

Compound	Calibration curve	R^2	Range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
ACV	y = 11.05x + 456.2	0.999	0.01–1	0.003	0.009
AMZ	y = 2.861x − 1639	0.998	0.6–20	0.3	0.6
AZT	y = 7.165x + 501.5	0.997	0.1–5	0.045	0.09
BPA	y = 6.945x + 40.50	0.999	0.01–1	0.003	0.009
CTZ	y = 15.51x + 383.7	0.998	0.01–1	0.003	0.009
CPA	y = 342.5x + 6987.	0.999	0.01–1	0.0015	0.003
CMT	y = 698.1x + 15942	0.999	0.01–1	0.0003	0.0009
CHD	y = 447.5x − 4951.	0.998	0.01–1	0.0009	0.0015
DLD	y = 19.22x − 78.04	0.999	0.05–5	0.01	0.03
DPH	y = 658.6x + 11577	0.998	0.05–5	0.01	0.03
EBT	y = 65.52x + 972.2	0.999	0.01–1	0.003	0.009
EPT	y = 4.522x + 55.89	0.999	0.1–5	0.03	0.09
FFD	y = 151.5x + 1236.	0.999	0.01–1	0.0009	0.0015
HXZ	y = 31.15x + 294.0	0.999	0.01–1	0.0015	0.003
KTF	y = 4.252x − 250.2	0.999	0.1–5	0.03	0.090
LTD	y = 291.9x + 4052.	0.999	0.01–1	0.0003	0.0009
OPD	y = 113.2x + 2306.	0.997	0.01–1	0.003	0.009
PMZ	y = 19.55x − 177.8	0.998	0.05–5	0.015	0.03
TFD	y = 11.43x + 142.4	0.999	0.01–1	0.003	0.009
TPD	y = 26.58x + 137.2	0.999	0.05–5	0.015	0.045

Table 3. Intra-day variation of six concentrations of 20 antihistamines

Compound	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
ACV	Precision (RSD[a], %)	9.2	3.5	3.7	2.5	3.0	0.1
	Accuracy (%)	98.7	106.9	96.8	102.9	99.2	100.5
AMZ	Conc. (μg/mL)	0.6	1	2.5	5	10	20
	Precision (RSD, %)	7.1	6.3	1.0	4.5	0.4	0.8
	Accuracy (%)	107.4	98.7	102.9	101.2	103.1	100.7
AZT	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	3.4	1.3	2.5	4.6	3.8	1.3
	Accuracy (%)	93.4	99.4	103.1	102.3	96.7	99.9
BPA	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.4	3.1	7.0	2.5	2.0	2.1
	Accuracy (%)	101.7	115.0	107.3	103.5	99.3	99.5
CTZ	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.2	5.2	8.5	4.3	0.9	3.3
	Accuracy (%)	103.7	112.5	97.8	105.3	102.9	100.2
CPA	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	8.1	5.6	5.4	1.0	4.3	1.0
	Accuracy (%)	87.0	105.5	114.3	101.7	100.5	98.3
CMT	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.9	9.1	7.1	4.5	0.5	4.1
	Accuracy (%)	98.3	105.2	97.5	106.4	98.4	99.7
CHD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	9.4	3.0	6.0	9.1	1.5	0.8
	Accuracy (%)	113.3	102.9	100.1	101.9	103.4	101.4
DLD	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	5.9	3.3	2.7	2.6	0.8	1.4
	Accuracy (%)	112.7	107.2	85.8	104.2	102.3	101.3
DPH	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	4.4	7.0	5.9	1.8	0.7	1.9
	Accuracy (%)	92.0	110.9	99.0	107.6	100.0	98.3
EBT	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	9.8	2.7	3.8	2.6	2.4	1.8
	Accuracy (%)	96.3	103.3	107.5	108.9	99.9	100.5
EPT	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	2.6	1.1	6.7	3.6	1.1	0.6
	Accuracy (%)	97.2	100.8	98.5	100.5	100.1	98.9
FFD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	6.3	2.2	9.2	4.5	3.6	1.3
	Accuracy (%)	102.7	107.2	107.2	102.8	102.3	101.1
HXZ	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	6.8	3.7	7.2	1.7	2.9	1.6
	Accuracy (%)	100.7	111.5	94.9	104.4	102.9	101.5

Table 3. Continued

KTF	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	7.4	8.5	2.9	7.5	3.1	0.3
	Accuracy (%)	108.7	96.1	98.2	101.1	101.8	100.5
LTD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	5.2	3.8	4.2	2.1	2.0	3.1
	Accuracy (%)	97.0	104.3	105.1	105.6	102.6	98.9
OPD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.1	4.1	5.7	1.4	2.2	5.1
	Accuracy (%)	99.0	105.3	99.6	107.2	101.9	100.3
PMZ	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	5.7	3.5	4.3	7.7	2.0	1.8
	Accuracy (%)	101.6	101.5	99.4	100.1	101.3	100.1
TFD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	5.7	1.6	5.8	4.0	3.1	1.8
	Accuracy (%)	100.7	114.0	98.1	106.9	100.3	99.7
TPD	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	3.3	0.2	1.0	5.2	2.5	3.8
	Accuracy (%)	107.7	101.5	103.4	102.1	100.1	100.0

[a)]RSD is defined as the standard deviation of a group of values divided by their mean

Table 4. Inter-day variation of six concentrations of 20 antihistamines

Compound	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
ACV	Precision (RSD[a)], %)	3.2	6.4	8.3	5.2	1.6	0.7
	Accuracy (%)	113.0	101.4	92.2	101.9	102.1	99.9
AMZ	Conc. (μg/mL)	0.6	1	2.5	5	10	20
	Precision (RSD, %)	1.5	6.2	3.0	2.1	2.6	2.8
	Accuracy (%)	100.9	108.0	101.8	102.8	105.7	101.9
AZT	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	5.9	1.6	1.9	1.2	4.2	0.5
	Accuracy (%)	98.9	100.9	103.7	99.2	98.5	100.6
BPA	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	6.2	8.4	7.1	0.9	1.6	1.3
	Accuracy (%)	99.3	107.2	105.5	106.3	97.2	99.6
CTZ	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	2.4	5.4	5.4	3.6	2.3	1.4
	Accuracy (%)	110.0	104.5	94.9	104.8	99.9	102.0
CPA	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	3.6	2.9	6.0	5.6	3.7	2.5
	Accuracy (%)	98.3	96.9	101.3	106.7	101.9	99.2
CMT	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	5.5	5.7	6.7	2.2	2.9	2.1
	Accuracy (%)	111.0	100.5	91.1	106.3	102.4	102.1

Table 4. Continued

CHD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.4	5.9	8.4	3.1	1.2	3.5
	Accuracy (%)	101.3	96.9	100.4	103.6	103.2	100.8
DLD	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	3.5	7.1	0.3	1.9	2.9	2.1
	Accuracy (%)	101.7	102.9	84.3	104.0	99.9	101.5
DPH	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	5.8	8.8	1.9	3.4	1.1	6.6
	Accuracy (%)	97.7	107.3	103.8	105.2	98.6	102.3
EBT	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	8.5	1.5	4.2	5.4	2.1	0.5
	Accuracy (%)	97.7	103.9	105.7	103.4	100.0	97.9
EPT	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	5.9	2.0	1.2	2.9	2.5	0.6
	Accuracy (%)	96.2	101.1	104.7	105.4	101.1	98.5
FFD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	6.8	3.7	7.2	1.7	2.9	1.6
	Accuracy (%)	100.7	111.5	94.9	104.4	102.9	101.5
HXZ	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	9.3	9.6	8.8	2.6	3.2	3.8
	Accuracy (%)	101.7	104.8	96.8	107.6	99.3	100.3
KTF	Conc. (μg/mL)	0.1	0.25	0.5	1	2.5	5
	Precision (RSD, %)	1.4	1.5	0.4	3.9	2.8	0.6
	Accuracy (%)	109.0	101.1	101.8	97.4	100.4	100.3
LTD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	7.1	2.2	8.9	1.3	0.5	1.4
	Accuracy (%)	97.0	104.3	105.1	105.6	102.6	98.9
OPD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	6.2	8.5	9.1	3.4	1.7	2.5
	Accuracy (%)	109.3	100.5	99.4	103.1	99.7	102.2
PMZ	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	3.4	1.4	4.0	3.9	1.9	2.2
	Accuracy (%)	105.2	99.1	98.9	102.0	101.3	100.5
TFD	Conc. (μg/mL)	0.01	0.05	0.1	0.2	0.5	1
	Precision (RSD, %)	4.7	4.5	2.9	8.6	2.2	0.5
	Accuracy (%)	110.0	107.9	100.4	103.6	99.9	101.6
TPD	Conc. (μg/mL)	0.05	0.25	0.5	1	2.5	5
	Precision (RSD, %)	4.9	0.9	1.4	5.7	2.7	0.4
	Accuracy (%)	106.1	100.9	102.1	108.4	100.2	99.7

[a)]RSD is defined as the standard deviation of a group of values divided by their mean

Table 5. Recovery of 20 antihistamines in dietary supplement samples

Compounds	Tablet (mean ± RSD[a]), %			Liquid (mean ± RSD), %			Pill (mean ± RSD), %			Powder (mean ± RSD), %			Capsule (mean ± RSD), %		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
ACV	99.8 ±0.4	96.8 ±1.2	105.1 ±1.3	94.7 ±0.8	96.7 ±0.9	92.7 ±0.7	105.1 ±2.6	103.2 ±1.9	107.4 ±1.2	108.9 ±2.2	98.3 ±1.7	102.73 ±0.8	95.1 ±2.7	91.1 ±4.9	90.8 ±5.0
AMZ	91.1 ±12	94.9 ±1.7	90.5 ±3.8	89.2 ±5.2	90.8 ±5.0	97.8 ±0.7	110.4 ±2.8	104.0 ±1.0	103.8 ±1.0	103.8 ±0.6	100.5 ±2.3	101.2 ±0.7	98.1 ±1.0	97.0 ±1.2	100.4 ±1.0
AZT	93.9 ±3.1	93.6 ±1.2	100.3 ±1.0	105.0 ±0.9	97.3 ±1.6	97.9 ±1.6	105.3 ±1.7	106.4 ±2.6	107.9 ±2.7	102.4 ±1.5	99.4 ±2.4	100.6 ±2.3	98.8 ±1.6	97.0 ±1.7	99.0 ±1.0
BPA	99.4 ±1.2	96.6 ±1.6	91.7 ±1.3	101.5 ±1.2	95.1 ±0.7	109.2 ±0.5	105.3 ±1.0	104.2 ±1.0	112.1 ±2.9	109.2 ±0.5	92.9 ±2.6	93.7 ±0.8	98.3 ±1.0	94.2 ±0.8	88.0 ±3.8
CTZ	98.0 ±2.2	99.6 ±0.2	98.1 ±1.7	96.7 ±1.3	105.1 ±0.9	97.6 ±1.1	88.5 ±1.3	109.8 ±1.6	116.1 ±0.8	88.0 ±3.8	91.3 ±1.6	95.2 ±0.7	99.4 ±1.0	97.2 ±1.6	97.6 ±1.1
CPA	98.7 ±1.2	100.7 ±0.5	101.1 ±0.8	99.0 ±1.0	98.9 ±1.2	97.7 ±2.1	108.2 ±0.9	114.9 ±1.1	104.1 ±1.0	93.5 ±2.7	102.0 ±0.6	98.9 ±1.7	97.7 ±0.7	90.4 ±2.0	91.9 ±1.6
CMT	98.2 ±1.3	113.7 ±1.0	99.3 ±3.7	97.3 ±1.1	99.8 ±1.2	101.5 ±1.2	110.4 ±2.8	103.0 ±1.7	95.8 ±0.9	100.3 ±0.1	94.9 ±0.8	97.7 ±0.7	92.5 ±4.4	104.5 ±0.9	103.7 ±0.9
CHD	97.2 ±1.2	106.1 ±0.8	107.7 ±0.5	98.0 ±1.9	94.3 ±0.7	99.5 ±1.9	91.7 ±1.1	103.9 ±1.9	107.8 ±5.0	107.7 ±1.2	101.5 ±0.8	101.2 ±1.1	94.2 ±2.0	95.5 ±0.6	108.5 ±1.1
DLD	98.5 ±1.4	97.4 ±0.6	102.4 ±1.5	97.9 ±1.6	98.8 ±1.2	97.2 ±1.7	109.5 ±0.8	103.1 ±1.5	100.4 ±0.3	100.8 ±0.4	103.6 ±1.1	97.4 ±1.5	97.9 ±1.8	97.4 ±1.3	103.7 ±0.9
DPH	99.0 ±1.5	99.9 ±0.2	100.3 ±0.4	98.9 ±1.2	98.3 ±1.0	104.4 ±0.2	97.7 ±1.0	94.0 ±0.6	97.4 ±0.3	99.9 ±0.1	117.9 ±1.6	99.3 ±1.8	92.9 ±2.6	92.5 ±0.8	97.2 ±1.1
EBT	111.3 ±0.5	105.2 ±0.6	103.5 ±0.7	104.8 ±0.6	100.3 ±1.7	96.9 ±1.6	102.9 ±3.0	96.7 ±1.2	94.2 ±1.5	101.7 ±0.8	84.8 ±0.2	93.2 ±0.7	97.1 ±1.7	97.2 ±1.7	98.9 ±1.1
EPT	104.1 ±0.6	102.5 ±0.4	95.8 ±1.1	88.5 ±2.3	100.4 ±0.4	100. 3±2.3	95.1 ±1.9	95.7 ±0.6	90.4 ±1.4	105.2 ±1.0	98.9 ±1.2	99.4 ±1.0	100.4 ±1.0	96.6 ±1.8	101.4 ±1.2
FFD	102.7 ±0.8	100.7 ±1.3	99.4 ±0.4	97.2 ±0.9	99.9 ±0.2	105.5 ±4.9	94.7 ±2.0	93.3 ±1.9	92.3 ±1.2	97.7 ±1.1	104.6 ±1.1	99.8 ±2.7	96.3 ±1.4	96.0 ±1.4	98.8 ±1.6
HXZ	100.3 ±2.3	97.2 ±2.0	101.2 ±0.7	105.6 ±0.8	102.4 ±1.5	95.4 ±0.8	95.0 ±1.9	91.9 ±1.2	93.8 ±1.2	100.4 ±1.0	99.0 ±1.0	97.1 ±1.7	97.8 ±1.0	90.3 ±2.6	94.9 ±0.8
KTF	94.7 ±2.6	97.4 ±0.9	96.8 ±3.4	99.3 ±1.3	88.5 ±2.3	100.9 ±0.5	115.3 ±3.3	98.3 ±1.3	96.5 ±0.6	98.6 ±1.4	94.9 ±2.2	93.7 ±0.8	91.9 ±1.6	90.8 ±5.0	91.5 ±5.0
LTD	107.9 ±2.8	108.9 ±2.2	97.0 ±1.7	102.3 ±0.9	109.7 ±0.5	98.6 ±1.7	100.2 ±1.2	107.7 ±1.1	104.8 ±1.0	90.8 ±5.0	91.2 ±4.9	89.4 ±5.2	95.8 ±1.1	102.2 ±0.4	101.5 ±1.1
OPD	96.4 ±0.8	99.9 ±0.1	95.4 ±1.5	98.5 ±0.3	100.5 ±1.2	93.8 ±0.8	104.7 ±1.0	108. 3±0.9	114.1 ±2.9	115.3 ±3.3	100.1 ±0.3	100.5 ±0.3	97.7 ±0.7	95.8 ±1.0	97.2 ±1.7
PMZ	101.5 ±0.9	99.4 ±2.4	100.6 ±2.3	109.7 ±0.5	100.0 ±1.3	99.8 ±0.9	97.4 ±0.7	88.5 ±1.3	94.2 ±1.5	94.3 ±0.8	93.7 ±0.8	92.9 ±2.6	100.5 ±0.8	97.9 ±2.4	101.9 ±0.6
TFD	97.9 ±1.7	105.6 ±0.8	91.6 ±4.2	100.7 ±1.3	101.2 ±1.1	105.2 ±2.7	98.2 ±0.5	93.5 ±1.6	95.4 ±3.8	96.5 ±0.8	97.1 ±1.7	98.8 ±1.2	97.2 ±0.9	101.3 ±1.0	103.7 ±2.2
TPD	99.3 ±1.3	100.6 ±1.5	98.4 ±0.7	99.5 ±2.9	97.7 ±3.4	100.3 ±2.1	100.1 ±0.2	89.3 ±0.6	100.3 ±01.0	89.4 ±5.0	95.3 ±1.0	102.8 ±3.0	99.6 ±0.1	96.5 ±0.3	114.6 ±0.3

[a]RSD is defined as the standard deviation of a group of values divided by their mean

Table 6. Stability of 20 antihistamines for 24 hours

Compound	Injection time (h)/Recovery (%)						Mean ± RSD[a] (%)
	0	2	4	6	12	24	
ACV	106.2	98.2	98.4	110.0	102.0	110.0	104.1±5.2
AMZ	106.0	112.0	102.0	99.4	104.0	112.0	105.9±4.9
AZT	94.2	108.0	103.2	103.6	110.0	102.8	103.6±5.3
BPA	95.0	105.0	105.2	102.8	95.0	98.8	100.3±4.7
CTZ	95.4	102.0	89.0	99.2	102.0	98.0	97.6±5.0
CPA	99.6	99.4	108.0	103.2	103.0	103.0	102.7±3.0
CMT	106.0	100.0	104.0	94.8	96.4	98.8	100.0±4.3
CHD	102.0	97.4	102.0	110.8	94.0	106.0	102.0±5.9
DLD	87.4	88.8	97.0	93.2	93.2	97.2	92.8±4.4
DPH	98.8	98.8	105.2	96.4	103.0	110.0	102.0±4.9
EBT	104.8	104.4	97.0	108.0	103.2	102.8	103.4±3.5
EPT	102.0	103.0	97.6	103.0	98.8	104.0	101.4±2.6
FFD	104.8	109.4	107.4	103.4	104.6	102.2	105.3±2.5
HXZ	106.8	105.0	104.0	99.6	99.0	102.8	102.9±3.0
KTF	90.2	85.4	88.0	83.6	88.6	95.2	88.5±4.6
LTD	94.4	106.4	103.6	99.4	95.0	98.8	99.6±4.7
OPD	93.4	103.0	96.4	93.0	94.4	97.5	96.3±3.9
PMZ	99.6	98.4	96.8	104.8	97.4	105.8	100.5±3.9
TFD	97.0	97.2	106.4	93.2	99.0	97.6	98.4±4.4
TPD	104.2	103.4	95.0	97.4	105.1	99.4	100.7±4.1

[a]RSD is defined as the standard deviation of a group of values divided by their mean

84.3-113.0% (inter-day accuracy).

3.2.4. Recovery

The recovery of method was determined by adding a known amount of target compounds using five replicates at three concentration levels (a low-concentration sample at approximately the LOQ level, a medium-concentration sample at ~10 times the LOQ level, and a high-concentration sample at ~50 times the LOQ level) in the dietary supplement samples. To evaluate the recovery of antihistamines, the peak areas of the pure standards and spiked samples were compared in five replicates. The mean of recovery of the 20 targeted compounds ranged from 90.5% to 113.7% for tablets, 88.5% to 109.7% for liquids, 88.5 to 116.1 for pills, 84.8% to 117.9% for powders, and 88.0% to 114.6% for capsules. The results are shown in *Table* 5.

3.2.5. Stability

The stability of method was evaluated by measuring freshly prepared standard solution of known amount for different period of time. Standard solution of target compounds was measured at several time points up to 24 h. The RSD values of the RSD values were less than 5.9% (*Table* 6).

4. Discussion

Previously, most of the simultaneous determinations of the antihistamines have been carried out only in the blood or urine samples from human; these methods can determine food or dietary supplements.

In this method, the chromatogram produced by LC-MS/MS, in addition MS/MS optimization was performed by the direct infusion and LC-MS/MS scans were performed using ESI operated in positive mode. The HPLC separation was achieved on a C_{18} reverse-phase column by using mobile phase of 0.1% formic acid in DW and 0.1% formic acid in acetonitrile of a gradient elution mode. The optimized method was validated for specificity, linearity, LOD,

LOQ, precision, accuracy, recovery and stability according to ICH guideline. The developed method was successfully applied to determine antihistamine compound in dietary supplements without any interference. The results demonstrated that the values were within the acceptable range.

5. Conclusions

A rapid, selective, and sensitive LC–MS/MS method was developed in this study to analyze 20 antihistamines intentionally adulterated in the dietary supplements. The analytical conditions of the LC–MS/MS method were optimized. The developed method was completely validated and found to perform satisfactorily for the qualitative and quantitative analyses of antihistamines. The method was applied for the analysis of dietary supplements to determine the concentration of illegal drugs.

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