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Sensitive liquid chromatography–tandem mass spectrometry method for the simultaneous determination of paracetamol and guaifenesin in human plasma

Xiaoyan Chen, Jia Huang, Zhang Kong, Dafang Zhong*

Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China

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Abstract

A rapid and sensitive method for the simultaneous determination of paracetamol and guaifenesin in human plasma was developed and validated, using high-performance liquid chromatographic separation with tandem mass spectrometric detection. After extracted from plasma samples by diethyl ether–dichloromethane (3:2, v/v), the analytes and internal standard osalmide were chromatographed on a C_{18} column. Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via atmospheric pressure chemical ionization (APCI). The method was linear in the concentration range of 0.05–20.0 μg/ml for paracetamol and 5.0–2000.0 ng/ml for guaifenesin. The intra- and inter-day precision was within 14% for both paracetamol and guaifenesin. The assay accuracy was within ±2.4% for the analytes. This is the first assay method described for the simultaneous determination of paracetamol and guaifenesin in plasma using one chromatographic run. The method was successfully employed in a pharmacokinetic study after an oral administration of a multicomponent formulation, containing 650 mg paracetamol, 200 mg guaifenesin, 60 mg pseudoephedrine and 20 mg dextrorphan.

Keywords: Paracetamol; Guaifenesin

1. Introduction

Paracetamol (acetaminophen, *N*-acetyl-*p*-amino-phenol) is widely used in the symptomatic management of pain and fever, and has been associated with liver necrosis in humans and experimental animals after high dose exposure [1,2]. Guaifenesin, 3-(2-methoxy-phenoxy)-l, 2-propandiol, is reported to reduce the viscosity of tenacious sputum and is used as an expectorant [3]. Paracetamol and/or guaifenesin is frequently combined with pseudoephedrine and dextrorphan for symptomatic treatment of coughs and the common cold. It has been reported that the combination of paracetamol with guaifenesin significantly increased the rate of paracetamol absorption availability [4,5]. Therefore, simultaneous quantification of these two drugs in human plasma was desired for pharmacokinetic studies, especially in the presence of other compounds.

Many methods exist for paracetamol quantification in plasma samples, including GC [6], GC/MS [7], and reversed-phase HPLC with UV [8–11]. Recently, a liquid chromatographic-tandem mass spectrometric (LC–MS–MS) method was also reported to determine simultaneously paracetamol and chlorpheniramine in human plasma, but the extraction recovery of paracetamol was only approximately 20% [12]. Compared with paracetamol, very few methods were reported for the determination of guaifenesin in plasma. Stavchansky et al. [13] and Aluri and Stavchansky [14] described HPLC methods to determine guaifenesin in human plasma, using 1-ml plasma sample. The chromatographic run time for one sample was more than 9 min. At the same time, none of these methods demonstrate the simultaneous quantification of these two drugs in biological fluids.

* Corresponding author. Fax: +86 24 23902539.
E-mail address: zhongdf@china.com (D. Zhong).

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The purpose of this study was to simplify, to speed-up and to assay simultaneously paracetamol and guaifenesin in human plasma using the liquid chromatographic–tandem mass spectrometric (LC–MS–MS) technique which can be used for pharmacokinetic studies after oral administration of multicomponent formulations, containing paracetamol, guaifenesin, pseudoephedrine and dextrorphan.

2. Experimental

2.1. Materials

Paracetamol (99.6% purity), guaifenesin (99.2% purity) and osalmide (internal standard, 99.5% purity) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) was purchased from Kangkede Chemical (Tianjin, China). Diethyl ether, dichloromethane and other chemicals (analytical grade) were from Shenyang Chemical Company (Shenyang, China). Blank (drug free) human plasma was obtained from Shenyang Blood Donor Service (Shenyang, China). Distilled water, prepared from demineralized water was used throughout the study.

2.2. Instrumentation

The LC–MS–MS system consisted of a Shimadzu series LC-10AD pump and SIL-HTA autosampler (Kyoto, Japan) and a Thermo Finnigan TSQ Quantum Ultra triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source. Data acquisition was performed with Xcalibur 1.3 software (Thermo Finnigan, USA). Peak integration and calibration were performed using LCQuan software (Thermo Finnigan, USA).

2.3. LC–MS–MS conditions

The chromatographic seperation was achieved on a Zorbax SB C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Wilmington, DE, USA) with a 4 mm × 3.0 mm i.d. SecurityGuard C_{18} (5 μm) guard column (Phenomenex, Torrance, CA, USA), using a mobile phase of methanol–water–formic acid (80:20:0.5, v/v/v), which was degassed by sonication before use. The liquid flow-rate was set at 0.6 ml/min. The column temperature was maintained at room temperature.

Mass spectrometer was operated in the positive mode. Quantification was performed using selected reaction monitoring (SRM) of the transitions of *m/z* 152 → 110 for paracetamol, *m/z* 199 → 125 for guaifenesin and *m/z* 230 → 121 for osalmide (I.S.), respectively, with a scan time of 0.3 s per transition. The tuning parameters were optimized for paracetamol, guaifenesin and I.S. by infusing a solution, containing 1 μg/ml of each analytes at a flow-rate of 10 μL/min into the mobile phase (0.5 ml/min) using a post-column "T" connection. The optimal MS parameters obtained were as follows: the corona discharge current was set at 4.0 μA with a source CID voltage of 10 V, the temperatures of the vaporizer and the heated capillary were 450 and 300 °C, respectively. Nitrogen was used as the sheath (35 Arb) and auxiliary (8 Arb) gas. Argon was used as the collision gas at a pressure of approximately 1.0 m Torr. The optimized collision energies chosen for paracetamol, guaifenesin and I.S. were 20, 15 and 25 eV, respectively. Fig. 1 shows the product ion mass spectra of $[M + H]^+$ of paracetamol, guaifenesin and I.S.

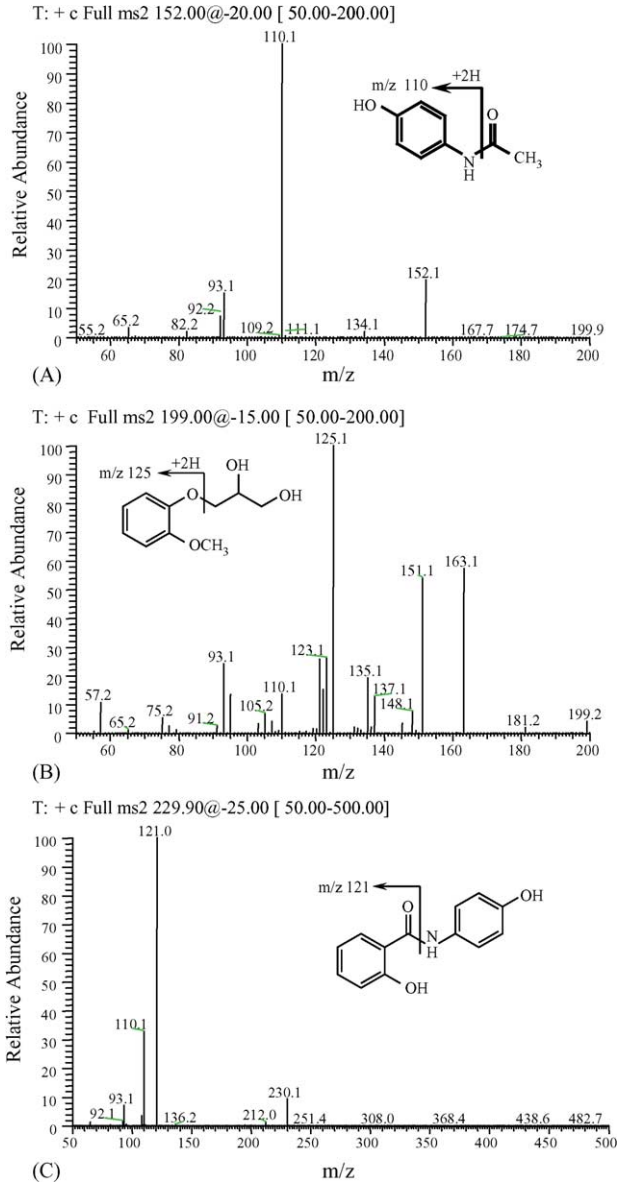

Fig. 1. Full scan product ion mass spectra of $[M + H]^+$ of paracetamol (A), guaifenesin (B) and osalmide (I.S., C).

2.4. Preparation of standard and quality control samples

Standard stock solutions of paracetamol and guaifenesin were prepared individually in methanol at the concentrations

of 400.0 and 40.0 μg/ml. A combined standard solution was prepared by adding 1.0 ml aliquots of each standard stock solution to a 10 ml volumetric flask and made up to volume with a mixture of methanol–water (50:50, v/v) to yield a solution with final concentrations of 40.0 μg/ml of paracetamol and 4.0 μg/ml of guaifenesin. The solution was then serially diluted with water to obtain the desired concentrations. I.S. working solution (1.0 μg/ml) was also prepared by diluting the 400 μg/ml stock solution of osalmide with water. All the solutions were stored at 4 °C and were brought to room temperature before use.

Calibration curves were prepared by spiking 50 μl of the appropriate standard solution to 100 μl of blank human plasma. Effective concentrations in plasma samples were 0.05, 0.1, 0.3, 1.0, 4.0, 10.0 and 20.0 μg/ml for paracetamol, and 5.0, 10.0, 30.0, 100, 400, 1000 and 2000 ng/ml for guaifenesin. The quality control samples (QCs) used in the validation and during the pharmacokinetic study were prepared in the same way as the calibration standards. The nominal plasma concentrations of QC samples were 0.1, 4.0 and 16.0 μg/ml for paracetamol and 10.0, 400 and 1600 ng/ml for guaifenesin. The spiked plasma samples (standards and quality controls) were extracted on each analytical batch along with the unknown samples.

2.5. Sample preparation

To a 100-μl aliquot of plasma sample, 100 μl of internal standard (1.0 μg/ml osalmide) and 50 μl of water were added. The samples were briefly mixed and 3 ml of a mixture diethyl ether–dichloromethane (3:2, v/v) were added. The mixture was vortex-mixed for approximate 1 min, then shaken on a mechanical shaker for 10 min. After centrifugation at 2000 × *g* for 5 min, the upper organic layer was removed and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The dry residue was reconstituted in 200 μl of the mobile phase, then vortex-mixed. A 20-μl aliquot of the resulting solution was injected onto the LC–MS–MS system for analysis.

2.6. Method validation

The method was validated for linearity, lower limit of quantification (LLOQ), accuracy and precision. To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on 3 separate days. Accuracy and precision were also assessed by determination of QC samples using six replicate preparations of plasma samples at three concentration levels (Table 1) for both paracetamol and guaifenesin on 3 validation days. Accuracy was expressed by relative error (R.E.) and precision by relative standard deviation (R.S.D.).

The lower limits of quantification (LLOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, were evaluated by analyzing samples which were prepared in six replicates as follows: spiking 50 μl of the standard solution, containing 100.0 ng/ml paracetamol and 10.0 ng/ml guaifenesin to 100 μl of blank human plasma.

Table 1
Accuracy and precision for the analysis of paracetamol and guaifenesin in human plasma (in prestudy validation, six replicates per day)

Concentration (ng/ml)		R.S.D. (%)		Relative error (%)
Added	Found (mean)	Intra-day	Inter-day	
Paracetamol				
100	102.4	3.7	6.1	2.4
4000	4027.8	6.6	11.9	0.7
16000	16082.3	7.3	6.0	0.5
Guaifenesin				
10.0	9.9	3.9	8.6	−0.6
400	400.3	3.2	14.8	0.1
1600	1593.8	6.5	11.2	−0.4

For the determination of recovery, blank human plasma was processed according to the sample preparation procedure as described above. The organic layer was evaporated to dryness, and dry extracts were reconstituted in the mobile phase adding appropriate standards at concentrations corresponding to the final concentration of the extracted plasma samples. These spike-after-extraction samples represented 100% recovery. The extraction recoveries of paracetamol, and guaifenesin were determined by comparing the mean peak areas of six extracted low, medium and high QC samples to mean peak areas of six spike-after-extract samples at the same concentrations. Recovery of I.S. was also evaluated by comparing the mean peak areas of six extracted medium QC samples to mean peak areas of six reference solutions spiked in extracted plasma samples of the same concentrations.

Stability of processing (three freeze-thaw cycles, benchtop for 2 h), chromatography (re-injection) and sample storage (−20 °C for 30 days) were assessed by analyzing replicates ($n = 3$) of QC samples (at the concentrations of 0.1 and 16.0 μg/ml for paracetamol, 10.0 and 1600 ng/ml for guaifenesin), which were exposed to different time and temperature conditions. The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.

2.7. Pharmacokinetic study

The method was applied to determine the plasma concentrations of paracetamol and guaifenesin from a clinical trial in which 22 healthy male volunteers received an oral dosage tablet (containing 650 mg paracetamol, 200 mg guaifenesin, 60 mg pseudoephedrine and 20 mg dextrorphan). Blood samples were collected before and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12.0 h post-dosing. Plasma was separated by centrifugation of the heparinized samples at 2000 × *g* for 10 min and were stored at −20 °C until analysis.

3. Results and discussion

3.1. Mass spectrometry

An LC–MS–MS method for the determination of paracetamol, guaifenesin and osalmide in human plasma was investigated. Firstly, the possibility of using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source under positive ion detection mode was evaluated during the early stage of assay development. Results showed that APCI could offer higher sensitivity for the analytes than ESI. Consequently, APCI was chosen as the source for further study.

Parameters were optimized in order to obtain more abundant protonated molecules of the analytes. Initially, the temperature of the heating capillary was set at 280 °C, an adduct molecule of guaifenesin $[M + H + NH_3]^+$ (*m*/*z* 216) was observed to be the most abundant fragment. When the temperature was increased to 300 °C, the full scan spectrum was dominated by protonated molecule $[M + H]^+$ (*m*/*z* 199) for guaifenesin. Under the same condition, the full scan spectra were also dominated by $[M + H]^+$ for paracetamol and osalmide.

The quasimolecular ions with *m*/*z* 152, 199 and 230 represent paracetamol, guaifenesin and osalmide, respectively. After collision-induced dissociation, the major fragment ions observed in each product spectrum were at *m*/*z* 110, 125 and 121, respectively. Additional tuning of the CID energy onto the transition of *m*/*z* 152 → 110 (paracetamol), *m*/*z* 199 → 125 (guaifenesin) and *m*/*z* 230 → 121 (osalmide) further improved the sensitivity. Therefore, they were selected for sensitive quantification of paracetamol, guaifenesin and osalmide.

3.2. Chromatography

The mobile phase with a high percentage of organics (80% methanol) provided low background noise, rapid separation and good peak shape. In positive ion mode, the presence of a low amount of formic acid in the mobile phase could improve the detection of the analytes, consequently, improve the sensitivity. Under the present chromatographic conditions, the run time of each sample was only 3.0 min, which is much shorter than that (9 min) in the HPLC method [13], which separated guaifenesin and I.S. from each other and from endogenous components. The retention times were 2.2, 2.4 and 2.7 min for paracetamol, guaifenesin and osalmide, respectively.

3.3. Method validation

3.3.1. Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Fig. 2 shows the typical chromatograms of a blank, a spiked plasma sample with paracetamol (100 ng/ml), guaifenesin (10 ng/ml) and I.S. (1.0 μg/ml), and a plasma sample from a healthy volunteer 2 h after an oral administration. There was no significant interference from endogenous substances observed at the retention times of the analytes. At the same time, due to the high selectivity of tandem mass spectrometry, only certain ion reactions were chosen to be monitored, there was no interference from pseudoephedrine and dextrorphan coadministered.

It was reported that paracetamol is primarily metabolized by conjugation to form paracetamol glucuronide (PG) and paracetamol sulphate (PS), respectively [15], which showed higher plasma concentrations in humans [16]. These conjugated metabolites might fragment to paracetamol in the LC–MS–MS interface and be falsely detected as paracetamol. During the early stage of method development, the potential interference of these conjugated metabolites was evaluated. After plasma samples were treated by solid-phase extraction, PS could be detected in plasma samples of all volunteers 3 h after an administration of multicomponent formulations by monitoring the transitions of *m*/*z* 232 → 152, whereas PG was not found by monitoring the transitions of *m*/*z* 328 → 152. The HPLC retention time of PS is 2.1 min. However, both conjugated metabolites of paracetamol, PS and PG were not observed after plasma samples were treated by liquid–liquid extraction. The results showed the conjugates of paracetamol were not extracted from plasma with organic solvents, which might be attributed to their high polar character and glucuronide conjugate gave few MS response under APCI conditions. Thus, we concluded that interference from the conjugated metabolites was not significant for this method.

3.3.2. Matrix effect

The possibility of a matrix effect caused by ionization competition between the analytes and co-eluents exists when using LC–MS–MS for analysis. To evaluate the matrix effect in the experiment, chromatographic peak areas of each analyte from the spike-after-extraction samples at low and high concentration levels were compared to the neat standards at the same concentrations. Percent nominal concentrations estimated were within the acceptable limits (94.2–103.1%) after evaluating six different lots of plasma. The same evaluation was performed on I.S. and no significant peak area differences were observed. Thus, ion suppression or enhancement from plasma matrix was negligible for this method.

3.3.3. Linearity of calibration curves and lower limits of quantification (LLOQ)

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range 0.05–20.0 μg/ml for paracetamol and 5.0–2000.0 ng/ml for guaifenesin in human plasma. Typical equations of the calibration curves using weighted ($1/x^2$) least squares linear regression were as follows: Paracetamol: $y = 1.60 \times 10^{-2} + 0.676x$, $r^2 = 0.9964$; Guaifenesin: $y = 5.88 \times 10^{-4} + 1.55 \times 10^{-4}x$, $r^2 = 0.9976$. Where y represents the ratios of paracetamol/guaifenesin peak area to that of osalmide and x represents the plasma concentrations of

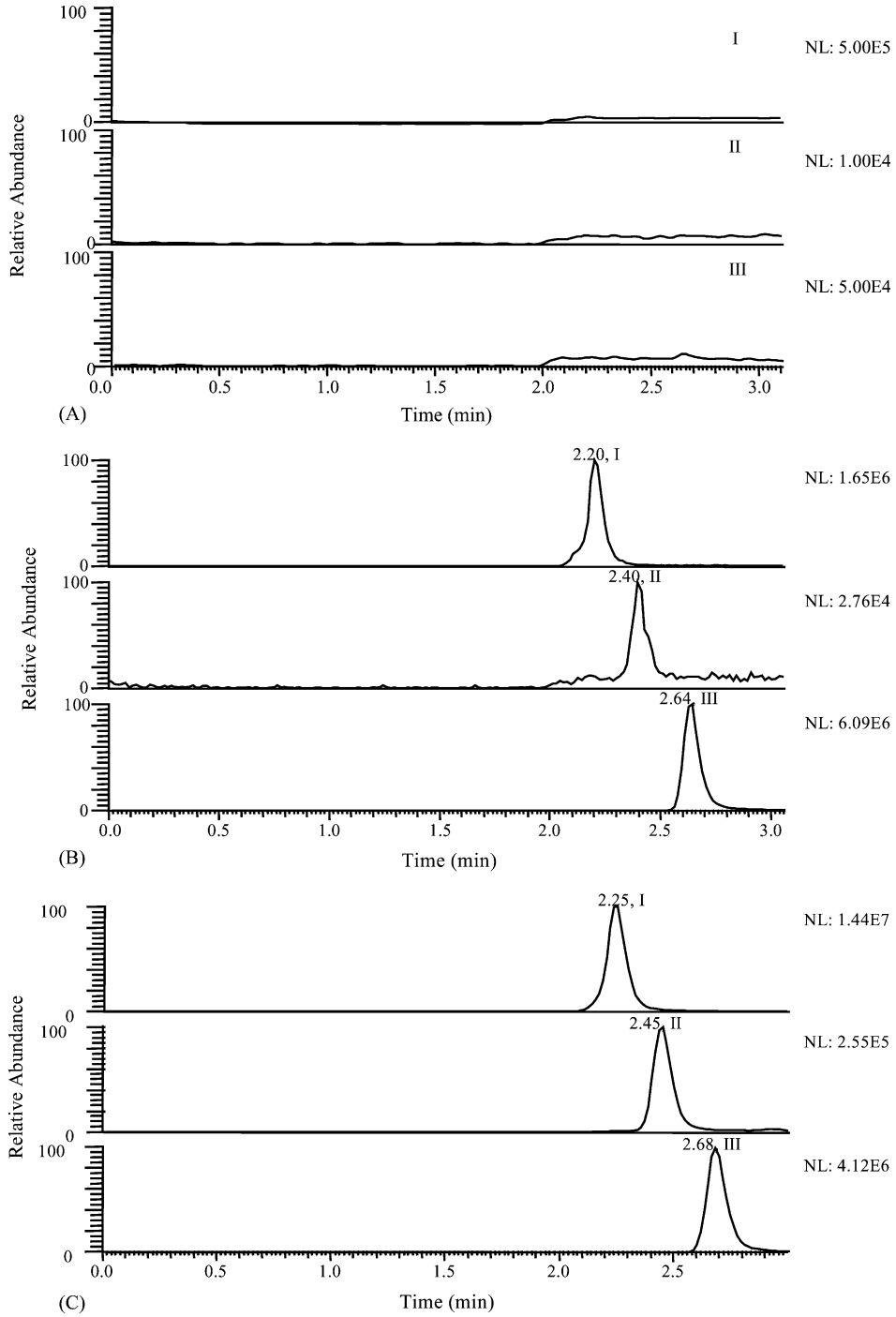

Fig. 2. Representative SRM chromatograms of paracetamol (I), guaifenesin (II) and I.S. (osalmide, III) in human plasma samples, (A) a blank plasma sample; (B) a blank plasma sample spiked with paracetamol (100.0 ng/ml), guaifenesin (10.0 ng/ml) and I.S. (1.0 μg/ml); (C) a volunteer plasma sample 2 h after an oral dose of 650 mg paracetamol, 200 mg guaifenesin, 60 mg pseudoephedrine and 20 mg dextrorphan.

paracetamol and guaifenesin. Good linearity was seen in the concentration ranges.

The lower limits of quantification were established at 50.0 ng/ml for paracetamol and 5.0 ng/ml for guaifenesin, which were sufficient for clinical pharmacokinetic studies following oral administration. The precision and accuracy values corresponding to LLOQ are shown in Table 2.

3.3.4. *Precision and accuracy*

Table 1 summarizes the intra- and inter-day precision and accuracy for paracetamol and guaifenesin evaluated by assaying the QC samples. The precision was calculated by using one-way ANOVA. In this assay, the intra-run precision was 7.3% or less for each QC level of paracetamol and 6.5% or less for each QC level of guaifenesin. The inter-run precision

Table 2
Accuracy and precision for paracetamol and guaifenesin at the plasma concentrations of LLOQ

	Concentration (ng/ml)		S.D. (ng/ml)	R.S.D. (%)	Relative error (%)
	Added	Found			
Guaifenesin	5.0	5.0	0.2	3.0	0.1
Paracetamol	50.0	51.0	1.2	2.3	1.9

was 11.9% or less for paracetamol and 14.8% or less for guaifenesin. The accuracy was within ±2.4% for paracetamol and within ±0.6% for guaifenesin. The above results demonstrated that the values were within the acceptable range and the method was accurate and precise.

3.3.5. Extraction recovery and stability

A simple one-step extraction was introduced to extract analytes from plasma. Using the low polar organic solvent and pH 7 resulted in high and consistent recoveries for all compounds. Mean extraction recoveries for paracetamol at 0.1, 4.0 and 16.0 μg/ml were 70.6, 70.3 and 74.4%, respectively. For guaifenesin, the recovery values at 10.0, 400 and 1600 ng/ml were 83.4, 85.8 and 82.2% ($n = 3$), respectively. Mean recovery for the internal standard (1.0 μg/ml) was 76.2% ($n = 3$). All recoveries had R.S.D. better than 4% throughout the entire standard concentration ranges, showing good consistency.

The results of stability experiments showed that no significant degradation occurred during chromatography, extraction and sample storage processes for paracetamol and guaifen-

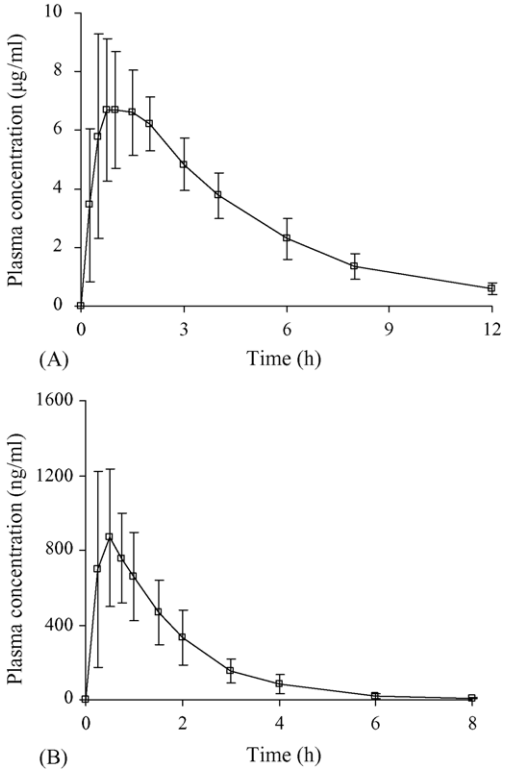

Fig. 3. Mean plasma concentration–time curves of paracetamol (A) and guaifenesin (B) after an oral administration of a multicomponent formulation, containing 650 mg paracetamol, 200 mg guaifenesin, 60 mg pseudoephedrine and 20 mg dextrorphan to 22 healthy volunteers (each point represents mean ± S.D.).

Table 3
Stability of paracetamol and guaifenesin in plasma samples ($n = 3$)

	Concentration (ng/ml)		S.D.	R.S.D. (%)	Relative error (%)
	Added	Found (mean)			
Three freeze-thaw cycles					
Paracetamol	100	105.8	3.0	2.8	5.8
	16000	15497	1450	9.4	−3.1
Guaifenesin	10.0	10.1	0.5	5.0	0.7
	1600	1742	21.5	1.2	8.9
Post-freezing (−20 °C)for 30 days					
Paracetamol	100	105.6	2.8	2.7	5.6
	16000	16637	109	0.7	4.0
Guaifenesin	10.0	10.9	0.1	1.2	8.7
	1600	1633	83.9	5.1	2.0
Post-treatment for 24 h (room temperature)					
Paracetamol	100	107.2	0.6	0.5	7.2
	16000	17422	187	1.1	8.9
Guaifenesin	10.0	10.3	0.6	6.0	3.4
	1600	1533	119	7.8	−4.2
Benchtop for 2 h (room temperature)					
Paracetamol	100	105	4.9	4.6	5.0
	16000	16396	1329	8.1	2.5
Guaifenesin	10.0	10.4	0.3	3.4	3.6
	1600	1567	75	4.8	−2.0

esin plasma samples. Stability data are shown in Table 3. In addition, standard stock solutions of paracetamol and guaifenesin were shown stable for at least 15 days at 4 °C.

3.4. Application of the method to pharmacokinetic study in healthy volunteers

This validated analytical method was used to study the pharmacokinetic profiles of paracetamol and guaifenesin in human plasma after an oral administration of a multicomponent formulation, containing 650 mg paracetamol, 200 mg guaifenesin, 60 mg pseudoephedramine and 20 mg dextrorphan. Profiles of the mean plasma concentration of paracetamol and guaifenesin versus time are shown in Fig. 3A and Fig. 3B. This simple and selective method for the determination of paracetamol and guaifenesin in human plasma was readily applicable to the clinical pharmacokinetic study for paracetamol and guaifenesin.

4. Conclusions

An LC–MS–MS method with APCI interface was developed and validated for the simultaneous determination of paracetamol and guaifenesin in human plasma. The method has significant advantage over other techniques used for measuring the two compounds in biological fluids. The major advantages of this method are the simple preparation, the rapidity of separation, the efficiency of analyzing two analytes simultaneously. Moreover, only 100 μl of plasma was required for the sample preparation procedures. This method was successfully applied to several pharmacokinetic studies for multicomponent formulations, containing paracetamol, guaifenesin and other components. In these studies, more than 120 samples were analyzed per day.

References

[1] J.A. Vale, A.T. Proudfoot, Lancet 346 (1995) 547.
[2] D.J. Miller, R. Hickman, R. Fratter, Gastroenterology 71 (1976) 109.
[3] D.G. Assimos, P. Langenstroer, R.F. Leinbach, N.S. Mandel, J.M. Stern, R.P. Holmes, J. Endourol. 13 (1999) 665.
[4] F. Perlik, I. Janku, V. Kordac, Int. J. Clin. Pharmacol. Ther. Toxicol. 26 (1988) 413.
[5] T. Dolezal, M. Krsiak, Naunyn Schmiedebergs Arch. Pharmacol. 366 (2002) 551.
[6] Y. Pegon, J.J. Vallon, Anal. Chim. Acta. 130 (1981) 405.
[7] S. Murray, A.R. Boobis, J. Chromatogr. 568 (1991) 341.
[8] A.G. Goicoechea, M.J. De Alda, J.L.L. Villa-Jato, J. Liq. Chromatogr. 18 (1995) 325.
[9] A. Esteban, M. Graells, J. Satorre, M. Perez-Mateo, J. Chromatogr. 573 (1992) 121.
[10] B.S. Nagaralli, J. Seetharamappa, B.G. Gowda, M.B. Melwanki, J. Chromatogr. B. 798 (2003) 49.
[11] M.A. Campanero, B. Calahorra, E. Garcia-Quètglas, A. López-Ocariz, J. Honorato, J. Pharm. Biomed. Anal. 20 (1999) 327.
[12] C. Celma, J.A. Alluè, J. Prunonosa, C. Peraire, R. Obach, J. Chromatogr. A. 870 (2000) 77.
[13] S. Stavchansky, S. Demirbas, L. Reyderman, C.K. Chai, J. Pharm. Biomed. Anal. 13 (1995) 919.
[14] J.B. Aluri, S. Stavchansky, J. Pharm. Biomed. Anal. 11 (1993) 803.
[15] L.F. Prescott, Br. J. Clin. Pharmacol. 10 (1980) 291S.
[16] J.S. Jensen, J. Valentine, R.W. Milne, A.M. Evans, J. Pharm. Biomed. Anal. 34 (2004) 585.