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Original Article

Simultaneous determination of paracetamol, 4-Aminophenol, 4-Chloroacetanilid, Benzyl alcohol, Benzaldehyde and EDTA by HPLC method in paracetamol injection ampoule

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Abstract

Paracetamol that is known as acetaminophen have the most consume as an analgesic and antipyretic drug in the world. That is formulated in single compound or mixture at many forms such as tablets, syrups, suspensions and drops. The last form is intravenous injections. Paracetamol derived from 4-minophenol which is synthesized by acylated the P-acetaminophenol and acetic anhydride. 4-aminophenol is the main impurity at manufacturing of paracetamol which could produce by hydrolysis during storage or synthesis under normal conditions (temperature, pH, etc.). Also, 4-chloroacetanilid may be observed as an impurity in the raw material of paracetamol synthesis. Benzyl alcohol is a preservative that used in Paracetamol for injection. It will be very important if there are analytical techniques to measuring paracetamol and its degradation products accurately and easily. Undoubtedly the most important and widely used, separation technique is chromatography. There are several reports about separation and quantitative determination of paracetamol lonely or simultaneous determination of paracetamol and 4-aminophenol. In this paper investigated simultaneous determination of paracetamol, 4-aminophenol, 4-chloroacetanilid, benzyl alcohol, benzaldehyde, and EDTA in paracetamol for injection ampoules by high performance liquid chromatography. By changing the ratio of mixing methanol and acetonitrile as mobile phase at the wavelength of 215 nm and pH=3 separation of all compounds were completely done.

Keywords: Paracetamol, Acetaminophen, Simultaneous determination of Paracetamol and related impurities, Analgesic and antipyretic, Acetaminophen with main impurities

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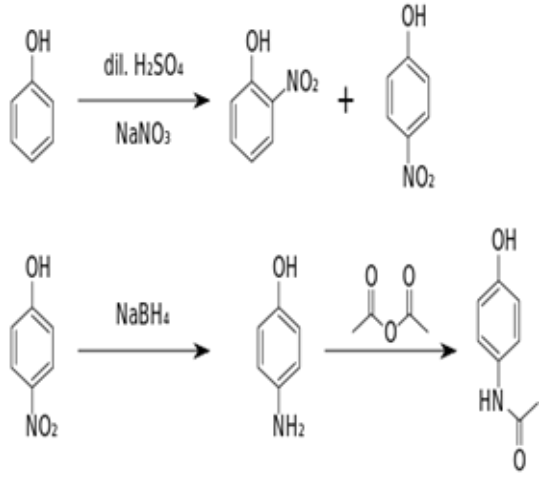
Introduction

Paracetamol has been using as medicine, widely. It is painkiller and reduces the temperature of patients with fever. These actions are known respectively as analgesic and antipyretic. In 1893, the white, odorless crystalline compound with a bitter taste that became known as paracetamol was discovered. This happened at University of Strasburg when Professor Adolf Kussmaul, Department of Internal Medicine, asked two young assistants, Arnold Cahn and Paul Hepp, to treat patients with naphthalene as it had been used elsewhere as an internal antiseptic.
The medicine had little effect on worms, but, paracetamol was found in the urine of patients who had taken phenacetin, who had a great reduction in fever temperature.
In 1887, the Bayer Company introduced the 4-ethoxy derivative, phenacetin, as a less toxic analogue of acetanilide. It was highly successful product and established the Bayer Company as a leading pharmaceutical manufacturer. Phenacetin has been using since 90 years ago and concerns over carcinogecity and kidney damaging properties. In 1889 it was demonstrated that paracetamol was a urinary metabolite of acetanilide. These discoveries, however, failed to attract much attention and were largely ignored at the time.
The use of paracetamol was first reported in 1893 and concluded that because of its hematological side effects of methaemoglobinaemia, it could not be recommended despite prompt antipyretic and analgesic actions. Brodie & Axelrod (1948) discovered that paracetamol was the main metabolite of both acetanilide and phenacetin, that paracetamol experienced a resurgence of interest. As a derivative of p-aminophenol, paracetamol corresponds to the active principal metabolite phenacetin (Suzen et al. 1998).
Their investigations showed that both acetanilide and phenacetin were metabolized into paracetamol and to which they owed their antipyretic and analgesic properties.
It was eventually ascertained that phenacetin had its own pharmacological action and was not dependent on paracetamol for its effects. Because a high proportion of phenacetin is converted into paracetamol in the liver, however, phenacetin required a large dosage to achieve any direct analgesic effect. In 1950 the first paracetamol product – a combination of paracetamol, aspirin and caffeine was on the United States market under the name Triagesic. Due to the report that Triagesic had been stricken with blood diseases, agranulocytoses, a sudden severe drop of white blood cell, it was immediately removed from the market.
Within a few years when became apparent that paracetamol had not connection with blood damage, by 1955 paracetamol was back in the American market. In 1956, 500 mg tablets of paracetamol went on sale in the United Kingdom and its popularity as an over-the-counter analgesic rapidly increased. This popularity was partly explained by the fact that paracetamol was proven to be easier on the stomach than some other analgesics (Monser et al. 2002; Dejaegher et al. 2008).
Synthesis In laboratory scale, paracetamol is prepared by a three-reaction sequence. First, nitration of phenol with sodium nitrate gives a mixture of two isomers, from which the wanted 4-nitrophenol (by~93 °C) can easily be separated by steam distillation. In this substitution reaction, phenol's oxygen is strongly activating, thus the reaction requires only mild conditions as compared to nitration of benzene itself. The nitro group is then reduced to an amine, giving4-aminophenol. This reaction can be accomplished using sodium borohydride. Finally, the amine is acetylated with acetic anhydride. The industrial process is analogous, but hydrogenation is used instead of the sodium borohydride reduction (Hawkins et al. 2007; Sornchaithawatwong et al. 2010).



Materials and Methods

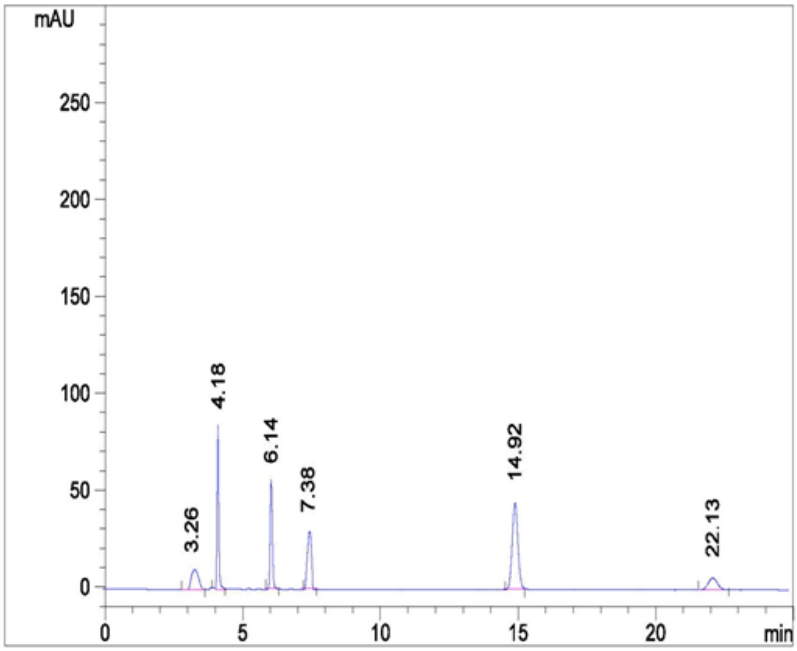
Apparatus a liquid chromatographic system consisted of an Agilent Isocratic LC pump 1200, with an automatic sample injection system (Agilent 1200 plus Auto sampler), Equipped with a Agilent 1200 detector. Chromatographic separation was performed on a MZ C18 reverse phase column packed with 5 μm Dimethyloctadecyl silane bonded (250 × 4.6 mm). All solutions were filtered through 0.45 μm Millipore filter prior to use and degassed using an ultrasonic bath. Chromatographic Conditions the mobile phase consisted of a mixture of methanol: acetonitrile: buffer phosphate (10:20:70; v/v) adjusted to pH 3.0 with 10% orthophosphoric acid. The mobile phase was prepared daily, filtered, and sonicated prior to use. All analyses were performed under isocratic conditions at a flow rate of 1 ml min-1 and the effluent was monitored at 215 nm. 20 μl of each solution was injected and chromatograms were recorded.

Chemicals and Reagents

Paracetamol STD (USP RS) Methanol and Acetonitrile was of HPLC grade, Triethylamin, Phosphoric acid, Potassium dihydrogen phosphate, EDTA, Benzaldehyde, 4-chloroacetanilid, 4-aminophenol, benzyl alcohol, purchased from Merck (Darmstadt, Germany). All other chemicals were commercial analytical reagent grade. Purified water was used for preparing mobile phase solutions. Standard Stock Solution Stock solutions were prepared separately by dissolving Paracetamol, EDTA, 4-chloroacetanilid, 4-Aminophenol, benzyl alcohol and Benzaldehyde in mobile phase to obtain concentrations of 1.0 mg ml-1. Monobasic potassium phosphate solution: Dissolve 2.72 g of monobasic potassium phosphate in water to make 900 mL and add slowly 2 mL triethylamin then adjust pH to 3 ± 0.1 with phosphoric acid and dilute to 1000 mL with water. Mobile phase: Prepare a filtered and degassed mixture of buffer, methanol, and Acetonitrile (70:10:20).

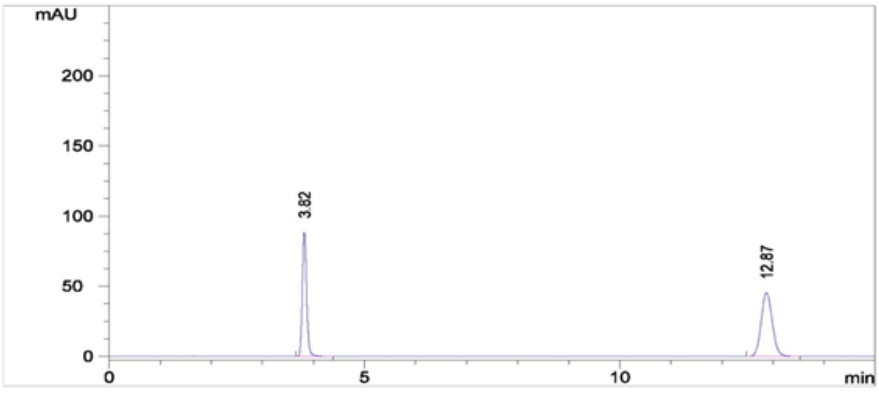
Results

Method optimization was demonstrated using a conventional HPLC system running in isocratic mode. A baseline separation was demonstrated on the 5 μm polar embedded phase for five process-related products and paracetamol in a run time window of 25 min (Fig.1). In contrast, the Dimethyloctadecyl silane bonded C18 column (250 x 4.6 mm) packed with 5μm particles sped up the analysis by more than three times and revealed a different separation and selectivity behavior (Fig.2). The limit of detection (LOD) for all compounds was in the range of 0.25μg/ml (Fig.5). The retention time reproducibility of the high speed isocratic was calculated with 5 runs to be in the range of 0.1-2 % RSD. The rapid analysis of a pharmaceutical product showed only a trace amount (0.02% w/w) of the degradation product 4-aminophenol.



#	Compound Name	Ret.Time	Area	Height	Width
1	EDTA	3.26	281.2	11.3	0.4
2	Acetaminophen	4.18	324.3	84.6	0.1
3	P-Aminophenol	6.14	336.3	56.2	0.1
4	P-Choloroaceta	7.38	441.7	28.7	0.2
5	Benzyl alcohol	14.92	646.2	44.6	0.3
6	Benzaldehyde	22.13	146.7	6.2	0.4

Figure 1: peak of standard



#	Compound Name	R.T	Area	Height	Width
1	Acetaminophen	3.82	341.5	91.8	0.1
2	Benzyl Alcohol	12.87	638.2	48.6	0.3

Figure 2: peak of Acetaminophen and benzyl alcohol

Table 1: Injection area of acetaminophen and benzyl alcohol

No.	Injection Con. μg/mL	injection1	injection2	injection3	Average1	RSD%
1	Con. 180 Acetaminophen	4257. 8	4258. 8	4258. 2	4258. 3	0. 0118
	Con. 108 benzyl alcohol	3605. 6	3606. 8	3603. 8	3605. 4	0. 0419
2	Con. 165 Acetaminophen	3904. 6	3891. 8	3801	3865. 8	1. 4611
	Con. 99 benzyl alcohol	3306. 5	3287. 5	3218. 3	3270. 8	1. 4192
3	Con. 150 Acetaminophen	3546. 1	3471	3558	3525. 0	1. 3382
	Con. 90 benzyl alcohol	2989. 6	2940	3007	2978. 9	1. 1671
4	Con. 135 Acetaminophen	3211. 1	3213. 1	3215. 1	3213. 1	0. 0622
	Con. 81 benzyl alcohol	2728. 4	2728. 7	2726. 3	2727. 8	0. 0479
5	Con. 120 Acetaminophen	2823. 4	2853. 2	2865	2847. 2	0. 7530
	Con. 72 benzyl alcohol	2389. 2	2421. 8	2428. 8	2413. 3	0. 8757

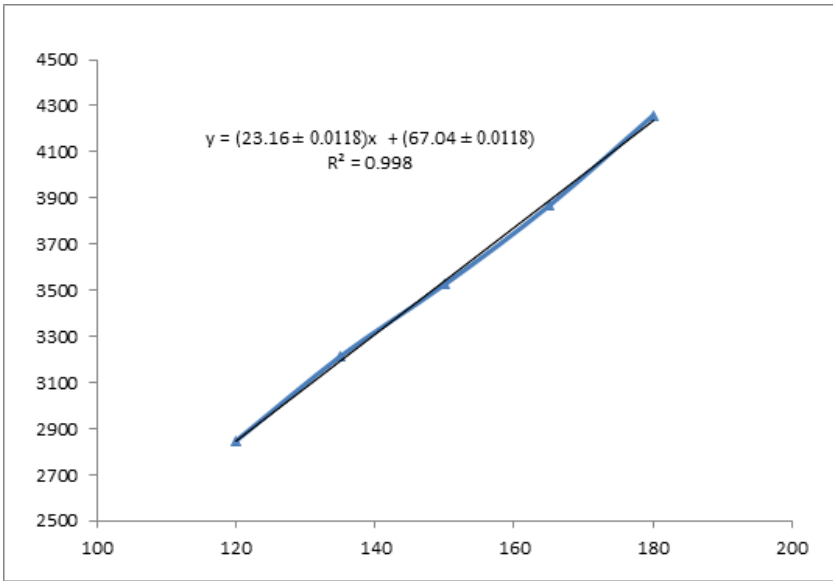


Figure 3: Calibration Curve of Acetaminophen

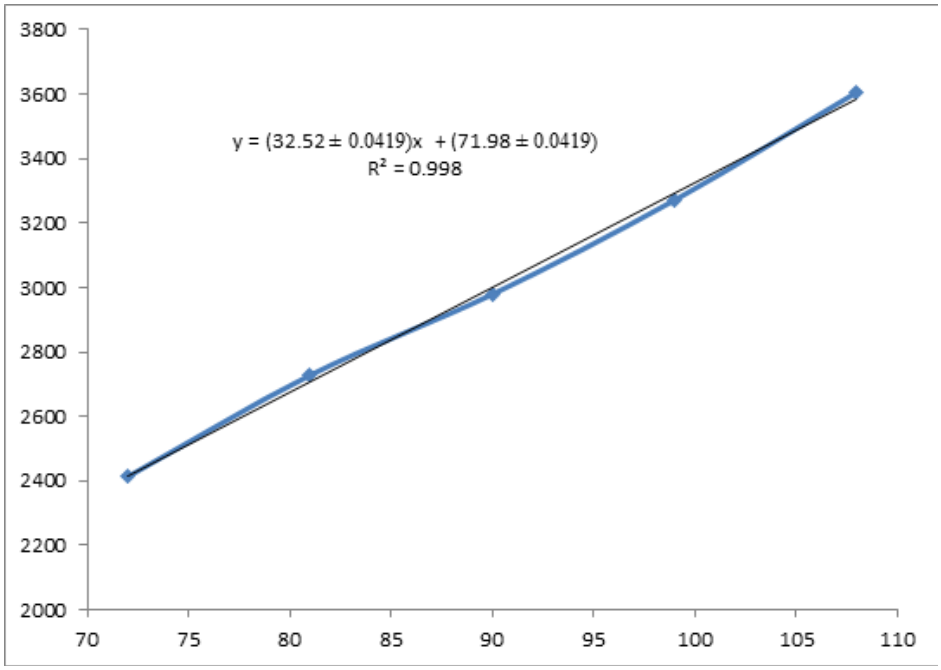
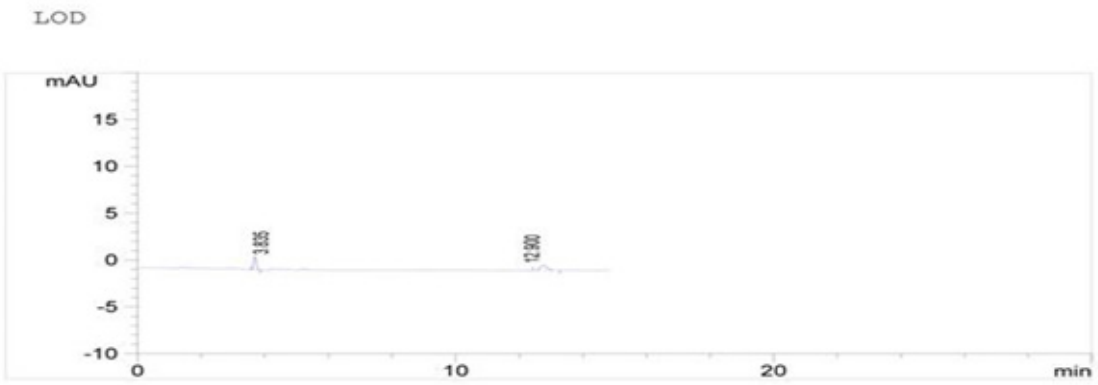


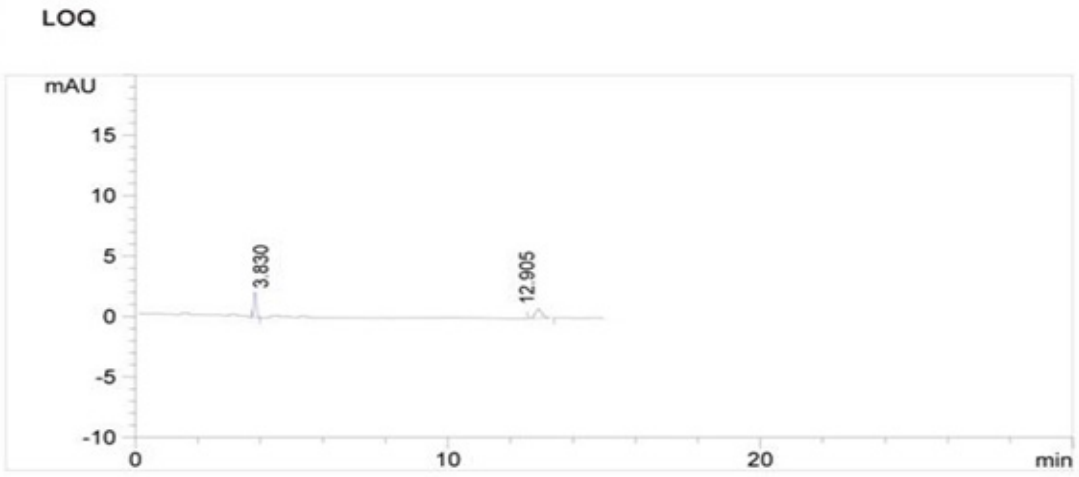
Figure 4: Calibration Curve of benzyl alcohol



#	Compound Name	R.T	Area	Area %	Height	Width
1	Acaetaminophen	3.83	5.7	30.9	0.3	0.1
2	Benzyl alcohol	12.90	4.8	29.1	0.2	0.2

C=120μg/mL	Area Std = 2847
C=0. 25 μg/mL	Area Sample = 6

Figure 5: LOD



#	Compound Name	R.T	Area	Area %	Height	Width
1	Acaetaminophen	3.83	11.7	50.9	2.1	0.1
2	Benzyl alcohol	12.91	11.2	49.1	0.8	0.2

C=120 μg/mL	Area Std = 2847
C= 0. 5 μg/mL	Area Sample = 12

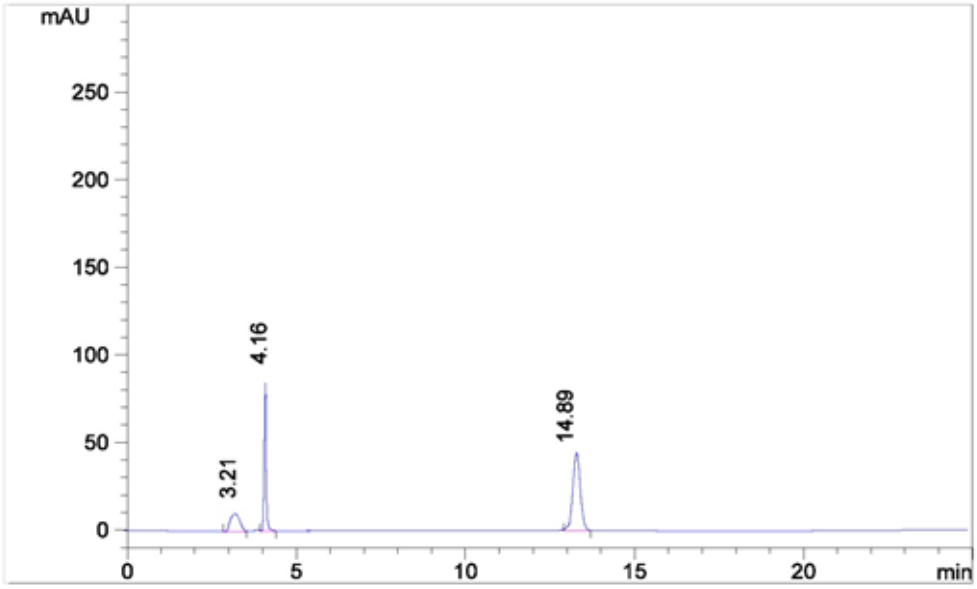
Figure 6: LOQ

Table 2: Recovery of Acetaminophen

C μg/mL	**150+10%(160)**	**150**
Area	3650.1 3653.3 3659.2	3851 3884. 2 3895. 2
Average area	3654.2	3876. 8

$$\text{Recovery}\,\% = \frac{A_{u1}}{A_{u2}} * \frac{C_{u2}}{C_{u1}} * 100$$

$$\text{Recovery}\,\% = \frac{3654.2}{3876.8} \times \frac{160}{150} \times 100 = 100.54\,\%$$



#	Compound Name	Ret.Time	Area	Height	Width
1	EDTA	3.21	286.9	11.3	0.4
2	Acetaminophen	4.16	328.5	84.6	0.1
3	Benzyl alcohol	14.89	641.8	44.6	0.3

Figure 7: peak of Acetaminophen ampoule

Discussion

Today, needs to new methods that can be faster, more sensitive and chipper due to production of new generation of drugs that contain paracetamol.

In this study we found a new HPLC method can determine simultaneous detection of paracetamol and its impurity that made from paracetamol degradation.

Conclusion

In the present study, we have shown that our proposed method using HPLC is convenient for simultaneous detection of acetaminophen, EDTA, 4-Aminophenol, 4-Chloroacetanilid, benzyl alcohol and benzaldehyde.

The results of this reliable HPLC method demonstrate a high level of precision and enough sensitivity for the determination of all six compounds simultaneously. The present HPLC method is simple, accurate and precise and can be used for the determination of Acetaminophen, 4-Aminophenol, 4-Chloroacetanilid and benzyl alcohol and possible degradation and impurity products in ampoule dosage forms. Thus, this procedure can be easily adopted for routine quality control analysis of ampoule dosage forms without any interference from the excipients or each other. Previously, several studies reported simultaneous detection of Acetaminophen and 4-Aminophenol and plus 4-Chloroacetanilid. Acetaminophen and its impurity product benzyl alcohol were also analyzed simultane-

ously using HPLC. However, acetaminophen and 4-Aminophenol, 4-Chloroacetanilid, EDTA and their impurity products plus benzyl alcohol have not been analyzed simultaneously until now. In some of the analgesics, Acetaminophen and benzyl alcohol exist together with EDTA. Therefore, our proposed method for simultaneous detection of these six substances is important for quality control laboratories where economy and time are essential.

Acknowledgements

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