Talanta 98 (2012) 220–225

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Operator care and eco-concerned development of a fast, facile and economical assay for basic nitrogenous drugs based on simplified ion-pair mini-scale extraction using safer solvent combined with drop-based spectrophotometry

Samarwadee Plianwong, Areerut Sripattanaporn, Kwanrutai Waewsa-nga, Parin Buacheen, Praneet Opanasopit, Tanasait Ngawhirunpat, Theerasak Rojanarata *

Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand

ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised form
22 June 2012
Accepted 25 June 2012
Available online 1 July 2012

Keywords:
Mini-scale extraction
Ion-pair
Drop-based spectrophotometry
Basic nitrogenous drugs
Chlorpheniramine maleate

ABSTRACT

A fast, facile, and economical assay for basic nitrogenous drugs has been developed based on the mini-scale extraction of the drug–dye ion pair complex combined with the use of safe-for-analyst and eco-friendlier organic extractant and drop-based micro-spectrophotometry. Instead of using large volume devices, the extraction was simply carried out in typical 1.5 mL microcentrifuge tubes along with the use of micropipettes for accurate transfer of liquids, vortex mixer for efficient partitioning of solutes and benchtop centrifuge for rapid phase separation. In the last step, back-extraction was performed by using the microvolume of acidic solution in order to concentrate the colored species into a confined aqueous microdrop and to keep the analyst away from unwanted contact and inhalation of organic solvents during the quantitation step which was achieved by using cuvetteless UV–vis micro-spectrophotometry without any prior dilutions. Using chlorpheniramine maleate as a representative analyte and *n*-butyl acetate as a less toxic and non-ozone depleting extractant, the miniaturized method was less laborious and much faster. It was accurate, precise and insensitive to the interferences from common excipients. Notably, it gave the assay results of drug in tablets and oral solution comparable to the large-scale pharmacopeial method while the consumption of organic solvents and the release of wastes were lowered by 200–400 folds.

1. Introduction

Liquid–liquid extraction is one of the methods used in the pharmaceutical analysis for the separation of the analyte from the formulation matrix. Because of simple equipment requirement, this technique is still used even though some substitute methods such as chromatography and solid phase extraction have emerged. In the British Pharmacopoeia and the United States Pharmacopeia (USP), solvent extraction remains a standard method for the assay of salts of organic nitrogenous basic drugs e.g. chlorpheniramine maleate (CPM) and brompheniramine maleate in the pharmaceutical preparations [1,2]. The principle is based on the extraction of drugs upon the pH adjustment into either aqueous or organic phase, followed by conventional UV spectrophotometry. Although the method is straightforward, the extraction procedures are conducted in the large volume using separatory funnels, thus requiring costly and massive amounts of organic solvents such as probably carcinogenic chloroform or highly flammable hexane. Moreover, it is laborious for a large number of samples and sometimes repeated extractions are required; thereby further increasing the analysis time, undesirable exposure to toxic solvents and the release of waste into the environment.

In the last decades, the global trend in chemical analysis has shifted towards simplifying, downsizing and greening sample preparation and analysis. Novel miniaturized liquid phase extraction has evolved from the more classical techniques and become attractive alternatives because of the simplicity, high extraction efficiency, short operation time and decreased volume of organic solvents consumed as well as wastes released [3]. Examples of these microextraction techniques include single-drop microextraction (SDME) [4–7], hollow-fiber microextraction (HF-LPME) [8–10], directly-suspended droplet microextraction (DSDME) [11–13] and dispersive liquid–liquid microextraction (DLLME) [14–17]. Nonetheless, some limitations exist in these methods. For instance, microdrop in SDME or DSDME is sensitive to gravity, shear force or speedy stirring and easily dislodged from the tip of microsyringe

* Corresponding author. Tel.: +66 34 255800; fax: +66 34 255801.
E-mail addresses: teerasak@su.ac.th, rtheerasak@yahoo.com (T. Rojanarata).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.06.075

needle; air bubbles are often formed and hydrophobic substances can be adsorbed on the surface of HF, reducing the transport rate and reproducibility of the extraction; at least two miscible organic solvents e.g. chloroform together with methanol are prerequisite for DLLME as the extraction solvents and disperser solvents [18,19]. In the aspect of operator safety and environmental compatibility, many previously reported microextraction methods still involved the use of unsafe, carcinogenic or ozone-depleting solvents such as chloroform and carbon tetrachloride, leading to the undesirable exposure to the analyst and possible release into the environment especially during the detection step where the analyte present in or diluted by such volatile solvents was manually measured by spectrophotometry.

From the aforementioned disadvantages of pharmacopeial large-scale extraction and some limitations of new modes of microextraction, a simplified mini-scale extraction has been developed as an alternative assay for basic nitrogenous drugs. Not only aiming to obtain a fast, facile and economical method with satisfactory analytical performance, the method design has deliberately emphasized on the use of procedures and reagents which were safe for the operator and friendly to the environment. Here, an extraction-spectrophotometric assay for a representative drug CPM in the pharmaceutical formulations has been proposed. The method was based on the downscaled extraction of the ion pair complex formed between the drug and anionic dye methyl orange (MO) using less toxic and non-ozone depleting *n*-butyl acetate as an extraction solvent and common laboratory equipments. After the colored species was finally back-extracted into a safer aqueous phase as a microdrop, the measurement was done by using commercial microvolume drop-and-measure UV–vis specrophotometer.

2. Experimental

2.1. Reagents and apparatus

CPM standard with 99.8% purity was obtained from Sigma-Aldrich (USA). All other chemicals were of analytical grade from Merck (Germany). Distilled water was used to prepare all solutions. A stock solution of MO was prepared at the concentration of 0.6 mg mL^{-1}. The commercial samples of CPM oral solution (2 mg mL^{-1}) and CPM tablets (4 mg per tablet) were supplied by local drug stores in Thailand.

For the absorbance measurement in microvolume (~3 μL), NanoVue Plus® cuvetteless drop-and-measure spectrophotometer (GE Healthcare, UK) was used. The optical path length was set at 0.5 mm. Micropipettes and Lo-retention® tips were of Eppendorf (Hamburg, Germany). Microfuge®16 (Beckman Coulter) was used for the centrifugation.

2.2. Methods

2.2.1. Simplified mini-scale extraction-spectrophotometric method

Into a series of 1.5 mL microcentrifuge tubes, 100 μL of standard CPM solutions (5, 10, 20, 30, 40, 50 μg mL^{-1} in water) or sample solution (~20 μg mL^{-1} CPM in water) and 50 μL of 0.6 mg mL^{-1} MO in 50 mM potassium hydrogen phthalate/NaOH buffer, pH 5.0 were accurately added by micropipettes and vortexed for 1 min. Two hundred and fifty microlitres of *n*-butyl acetate was added, and the mixture was then vortexed for 2 min and centrifuged at 12,000 rpm for 1 min to separate into two phases. The upper organic phase portion in the volume of 230 μL was transferred by using micropipette to a new tube. The remaining mixture was extracted again with another 250 μL of *n*-butyl acetate in the same manner and 250 μL of organic phase was transferred to combine with the first portion. To the collected organic phase, 20 μL of 2 M hydrochloric acid was added and the mixture was vortexed for 2 min and centrifuged at 12,000 rpm for 1 min to separate into two phases. This resulted in the formation of a red microdrop at the bottom of the tube which could be transferred by the aid of micropipette and tip for the absorbance determination. The measurement was done by placing a drop of about 3 μL of the red aqueous solution on a pedestal of a cuvetteless drop-based spectrophotometer and reading the absorbance values at 510 nm, against a reagent blank similarly prepared without drug. A standard calibration curve was constructed or regression equation was derived to calculate the amount of analyte drug in the unknown samples.

2.2.2. Sample preparation for the proposed method

For tablets, the content of twenty tablets were weighed and ground into fine powder. An accurately weighed portion of the powder equivalent to 4 mg CPM was transferred into a 100 mL volumetric flask and dissolved with distilled water. The flask was subjected to sonication for 5 min and the volume was made up to 100 mL with water and mixed. Then, an aliquot of sample solution was centrifuged to precipitate the insoluble excipients and a clear supernatant was collected and further diluted to get a working concentration of about 20 μg mL^{-1}. For oral solution, a stock solution of sample was directly prepared by pipeting a suitable volume of the oral solution and diluting with water to the concentration of about 20 μg mL^{-1}.

2.2.3. Method validation

The analytical performance characteristics of the proposed method were validated according to ICH Q2 guideline [20] including the linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision and specificity.

3. Results and discussion

3.1. Method development

3.1.1. Principle and characteristics of the reaction

In the proposed method, the assay lies in the ion-associated reaction between CPM and the anionic dye MO. Under the assay condition where the pH was set at 5.0, the nitrogenous CPM drug was protonated and present in a positively charged form while MO that contained the sulphonphthalein group was mainly negatively charged. The ionic charge interaction resulted in the formation of yellow lipophilic ion-pair complex with the maximum absorption at 415 nm which was extractable by a suitable organic solvent. Upon the addition of acid, MO in the complex was further protonated at N atom and the ion-pair complex turned into red polar species. Thus, it could be back-extracted into the aqueous phase and its color intensity which related to the initial CPM concentration in the sample and could be determined spectrophotometrically at 510 nm. A conceptual principle of the proposed assay is illustrated in Fig. 1. In the studies, the stoichiometry of the ion-associates between CPM and MO was investigated by preparing the reaction solutions containing CPM and MO in varied molar ratio according to Job's method of continuous variation [21]. The results indicated that drug formed the complex with dye at the ratio of 1:1 (Fig. 2). In terms of the stability of red complex formed in the aqueous acid solution, it was found that the color remained constant for more than 24 h at the ambient condition, thus it was an appropriate species to be measured in the analysis.

3.1.2. Incorporation of operator care and eco-concern

To overcome the drawbacks of the pharmacopeial large-scale extraction and aforementioned limitations of microextraction techniques, a fast, simplified and cost-effective alternative method was developed with the incorporation of the operator care and

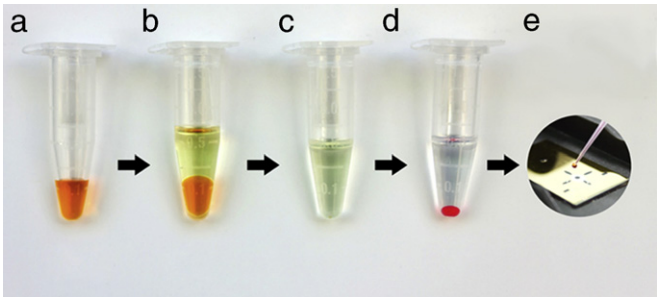

Fig. 1. Schematic representation of the proposed simplified ion-pair mini-scale extraction for the quantitative analysis of salt of basic nitrogenous drugs. The assay starts with the formation of the ion-pair complex between the drug and anionic dye (a) which is then extracted by the organic solvent (b). After the organic phase is collected in a new tube (c), microvolume of acid solution is added to back-extract the complex into the aqueous phase as a sedimented microdrop (d) which is then subjected to the absorbance measurement using drop-base spectrophotometer (e) with no dilution.

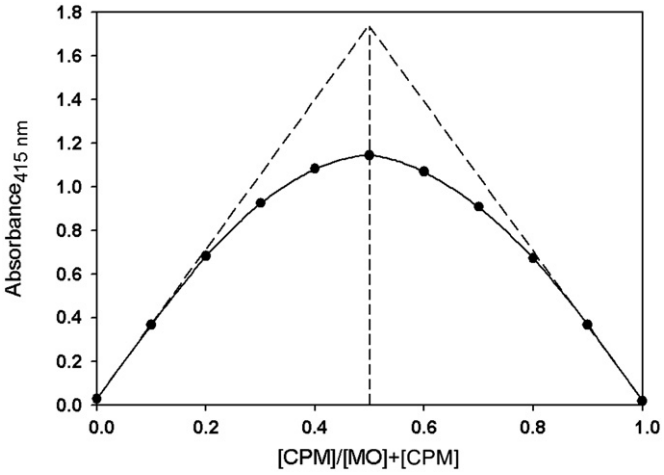

Fig. 2. Job's method of continuous variations plot for CPM–MO ion-pair complex formation. The absorbance values were measured in *n*-butyl acetate phase at 415 nm.

eco-concerned basis. This emphasized on the use of procedures and reagents which were safe for the analysts and friendly to the environment at the minimized quantities. In place of using large-volume separatory funnels, the extraction was simply carried out in inexpensive commercial microcentrifuge tubes along with the use of micropipettes for accurate measurement and transfer of liquids. While most previously reported microextraction methods used screw-capped vials or conical tubes with the size ranging from 4 to 50 mL, we found that 1.5 mL microcentrifuge tubes were efficient and appropriate devices for mini-scale extraction since they not only fitted the reagent-saving purpose and lessened the work space, but also provided the ease of vigorous shaking for efficient partitioning of solutes by the use of vortex mixer and fast phase separation between the organic and aqueous layers by the use of benchtop centrifuge. Moreover, their tightly closed lids well prevented the organic solvent and its vapor from leaking to the environment.

Compared to DLLME, the most similar mode of microextraction where the analyte is finally extracted into the sedimented phase, the proposed method was simpler as single organic solvent is needed as an extractant. Here, *n*-butyl acetate was chosen because it is not too volatile and it has acceptable extraction capability. From our study, the distribution of more than 95% of the CPM–MO complex from aqueous into *n*-butyl acetate was achieved after two rounds of extraction. For the safety issues, *n*-butyl acetate is not a potential or probable carcinogen like chloroform and carbon tetrachloride. Brief contact with skin is essentially non-irritating. Although liquid and vapor may cause mild discomfort, it has very low toxicity if swallowed. In the view of environmental concern, *n*-butyl acetate is not ozone-depleting and not classified as "dangerous to the environment" substance. The bioconcentration potential is low and it is readily biodegradable [22].

Unlike most previously reported microextraction methods where the analyte were still in or further diluted by organic solvents for the detection, the proposed method has been deliberately designed to back-extract the target species into an aqueous phase in order to keep the analysts away from unwanted contact of the organic solvent and inhalation of its vapor. In addition, the back-extraction step by using a microvolume of aqueous solution assisted to concentrate the colored species in a resulting confined microdrop of which the absorbance could be determined with ease by modern drop-and-measure spectrophotometer. This was easily done by adding diluted HCl to transform the lipophilic complex into a polar complex which was extractable into the aqueous phase. Since the concentration of CPM and MO used as well as the volume of HCl extractant added were elegantly chosen, the red microdrop formed after the last extraction step had an optimal color intensity which could be immediately measured by using cuvetteless microvolume spectrophotometer without any dilutions.

3.1.3. *Optimization of the mini-scale method*

Experimental parameters that can affect the formation and extractability of the ion-pair were optimized independently. These studies aimed to obtain the assay method with satisfactory analytical performance along with the reagent- and time-saving features.

3.1.3.1. Optimal pH. The pH influence on the formation of CPM–MO ion-pair complex was studied by setting up the reactions consisting of 20 μg mL^{-1} CPM solutions and 0.6 mg mL^{-1} MO in various buffers i.e. KCl–HCl (pH 1.5, 2), potassium hydrogen phthalate–HCl (pH 3, 4), potassium hydrogen phthalate–NaOH (pH 4.5, 5) and potassium hydrogen phosphate–NaOH (pH 6, 7.5). After the yellow ion-pair complex was formed, it was extracted into *n*-butyl acetate and measured for the absorbance at 415 nm. From the results, the maximum absorbance value which indicated the most favorable pH condition was observed over the range of

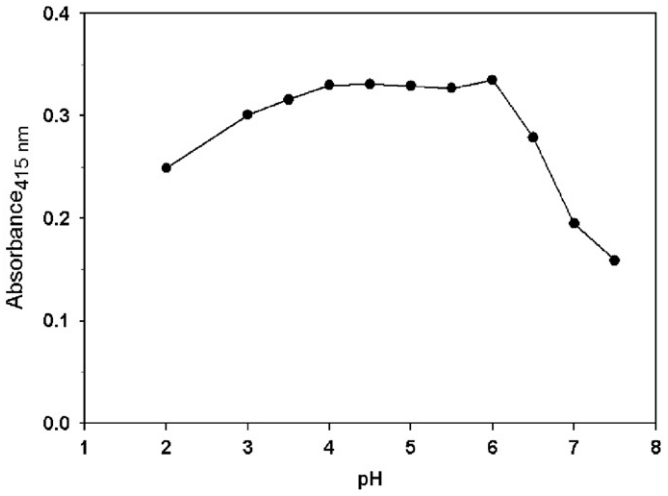

Fig. 3. Effect of pH on the ion-pair formation. The absorbance values were measured in *n*-butyl acetate phase at 415 nm.

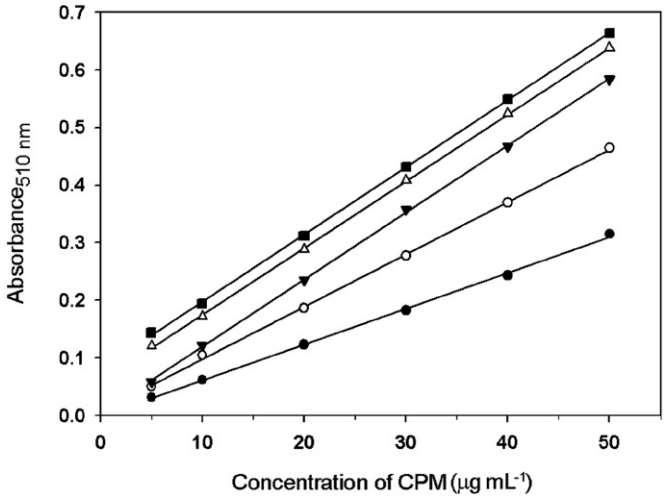

Fig. 4. Effect of volume of methyl orange solution (0.6 mg/ml): 10 μL (●), 25 μL (○), 50 μL (▼), 100 μL (Δ) and 150 μL (■).

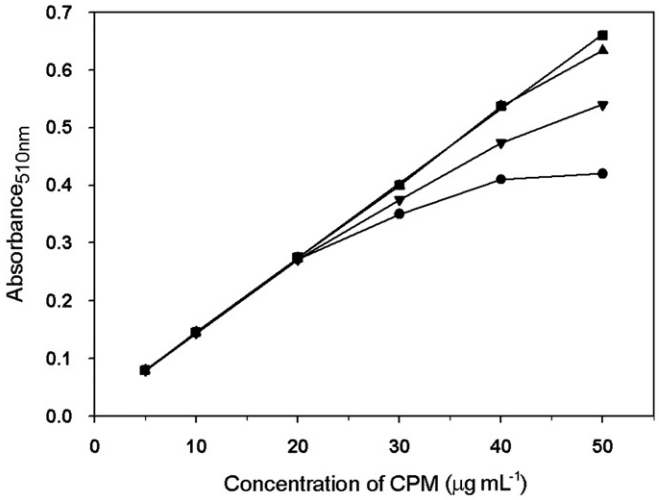

Fig. 5. Effect of concentration of HCl used to add in the back-extraction step at the fixed volume of 20 μL: 0.25 M (●), 0.5 M (▼), 1 M (▲) and 2 M (■).

pH 4–6 (Fig. 3). Therefore, the potassium hydrogen phthalate–NaOH buffer, pH 5.0 was used throughout the experiments.

3.1.3.2. Optimal quantity of MO solution. To investigate the optimal amount of MO, different volumes of 0.6 mg mL^{-1} dye solution were used in the reactions for the construction of the standard curves over the CPM concentration range of 5–50 μg mL^{-1}. It was observed that the use of 50 μL of MO solution gave the standard curves with the steepest slope which represented the highest sensitivity of the method. The use of double or triple amount of this level slightly gave higher absorbance values but did not further increase the slope or sensitivity (Fig. 4). Thus, 50 μL of 0.6 mg mL^{-1} MO was effective and sufficient amount required for the assay.

3.1.3.3. Optimal numbers of n-butyl acetate extraction. After the CPM–MO complex was formed under the optimal condition, it was extracted with a number of 250 μL portions of *n*-butyl acetate and the extraction efficiency in each step was evaluated by the absorbance measurement of yellow lipophilic complex at 415 nm. It was found that, after two rounds of extraction, more than 95% of the complex could be distributed from aqueous into the combined organic phase (data not shown). Hence, the extraction of ion-pair complex using two portions of 250 μL of *n*-butyl acetate was adequate and used in the standard procedure.

3.1.3.4. Optimal quantity of HCl for back-extraction. Since HCl was used to extract the ion-pair complex back into the aqueous phase and the resulting microdrop was supposed to have the appropriate color intensity for direct absorbance measurement without any prior dilutions using drop-and-measure UV–vis micro-spectrophotometry, the optimal concentration and volume of HCl were examined. It was found that the use of 0.25–1 M HCl in a volume of 20 μL gave non-linear standard curves, especially in the range of high concentrations of CPM, probably due to inadequate amount of acid for the complete protonation and thus reduced the extractability of ion-pair complex into the aqueous phase (Fig. 5). However, using 20 μL of 2 N HCl was sufficient for the complete extraction of the complex as seen from the negligibly small absorbance value of the aqueous microdrop obtained after the remaining organic phase was re-extracted with another quantity of acid. In addition, the absorbance of the red microdrop formed fell within the linear range of the NanoVue Plus® spectrophotometer.

Table 1
Linearity, LOD and LOQ of the proposed method.

Parameters	Proposed method
Linearity range (μg mL^{-1})	5–50
Regression equation	$A=0.0116C+0.0037$
Regression coefficient	0.9998
Slope (*a*)	0.0116
Y-intercept (b)	0.0037
LOD (μg mL^{-1})	0.76
LOQ (μg mL^{-1})	2.32

3.2. Analytical performance and reagent-saving feature

Once the optimal method protocols were established, a set of assays to verify its overall performance was carried out. As shown in Table 1, an excellent linear response of absorbance in relation to the concentration of CPM was found over the range of 5–50 μg mL^{-1} with the regression coefficient of 0.9998. The linear equation relating *A* (absorbance) to *C* (concentration, μg mL^{-1}) was $A=0.0116C+0.0037$. LOD and LOQ of proposed method as determined from calibration curve were 0.76 and 2.32 μg mL^{-1}, respectively.

The accuracy of the proposed method was determined by spiking three different levels of standard CPM into the sample solution which were prepared at the concentration of 20 μg mL^{-1} from tablets or oral solution. It was found that the percentage of recovery values were satisfactory in the range of 98.05–101.70% (Table 2). The precision of the proposed method, expressed as % RSD was found to be 0.87–0.94% for the repeatablility (intra-day precion) and 0.16–0.46% for the intermediate precision (inter-day precision) (Table 3). Since both % RSD values were less than 2%, the method was acceptably precise.

To investigate the specificity of the proposed method, the interference liabilities were carried out to explore the effect of common excipients that might be added during formulations. Samples were prepared by mixing known amount (20 μg mL^{-1}) of the drug and the excipients: sucrose, saccharin sodium, citric acid, methyl paraben, propyl paraben and sodium benzoate at the level commonly used in syrups and the recovery values were determined. As shown in Table 4, no interference was found from

Table 2
Accuracy of proposed method.

Formulation	CPM spiked ($\mu g\ mL^{-1}$)	CPM recovered ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%)
Tables	15	15.06	100.37	0.94
	20	20.03	100.16	1.42
	25	25.15	100.06	1.13
Syrups	15	15.07	100.47	1.23
	20	19.79	98.97	0.94
	25	24.77	99.07	1.14

Number of measurements (n) in each concentration level was equal to 3.

Table 3
Precision of proposed method.

Parameter	Tablets		Syrups	
	Labeled amount (%)	RSD (%)	Labeled amount (%)	RSD (%)
Intra-day[a]	100.33	0.94	100.83	0.87
Inter-day[b]	100.43	0.16	100.39	0.46

[a] Number of measurements (n) was equal to 6.
[b] Number of measurements (n) was equal to 3.

Table 4
Interference studies of the excipients in pharmaceutical formulation.

Excipients	Concentration added ($\mu g\ mL^{-1}$)	% Recovery
Magnesium stearate	6	99.8
Lactose	500	99.4
Talc	60	99.6
Sucrose	5000	99.8
Methyl paraben	60	100.2
Propyl paraben	60	99.0
Citric acid	60	100.2
Sodium benzoate	60	100.0
Saccharin sodium	60	99.8

Number of measurements (n) in each concentration level was equal to 3.

these excipients and the recovery values were 99.0–100.2%. This indicated the specificity of the method and the absence of interference from these excipients.

Besides the verification of the analytical performance, the reagent-saving feature and environmental compatibility of the proposed method was evaluted from the consumption of organic solvents. As determined per one sample extraction, only 0.5 mL of *n*-butyl acetate was used in the mini-scale method. This amount was dramatically lower than that consumed by the USP large-scale methods which employed 120 or 240 mL of hexane and chloroform for the assay of tablets or oral solution, respectively. Furthermore, as previously discussed, the solvent used in the proposed method was safer for health and eco-friendlier. Therefore, the mini-scale method not only offered the benefits in saving costs on chemical purchase and waste management, but it was also benign to the operators and the environment.

3.3. Application to the analysis of CPM in real samples

To demonstrate the applicability of the mini-scale extraction approach, the proposed method was tested for the assay of CPM in commercial tablets and syrups and the USP method [2] was used as a reference method for the comparison. As seen from the results in Table 5 and confirmed by statistical analysis (t-test) at 95% confidence level, there was no significant difference of the % labeled amount obtained from the proposed and the reference method.

Table 5
Results of CPM assay in pharmaceutical formulations obtained from the proposed and USP method.

Formulation	Proposed method	USP method
CPM tablets		
Labeled amount (%)	100.66	100.76
RSD (%)	1.20	1.66
CPM syrups		
Labeled amount (%)	101.35	101.81
RSD (%)	1.22	0.48

Number of measurements (n) was equal to 6.

4. Conclusion

This paper presents a fast, facile and economical alternative assay for salts of basic nitrogenous drugs using CPM as a model analyte. The method was delicately designed based on the use of unsophisticated and inexpensive equipments in parallel with the operator care and eco-concern to overcome the drawbacks of the pharmacopeial large-scale extraction and some limitations of modern microextraction techniques. Lying in the ion-pair extraction in a mini-scale with safer solvent, followed by drop-based micro-spectrophotometry, the developed method showed not only elegant analytical performance, but also satisfactory reagent-saving, safe-for-analyst and environmentally friendly features. Hence, this approach is an applicable alternative for routine quality control of basic nitrogenous drugs and considered as a green paradigm for the development of sustainable pharmaceutical and chemical assays.

Acknowledgments

Financial support from Department of Environmental Quality Promotion, Ministry of Natural Resources and Environment, Thailand Toray Science Foundation, New Charoen Pharm Co., Ltd. and Faculty of Pharmacy, Silpakorn University are gratefully acknowledged. Also, the authors are very thankful to Eugene Kilayco for his valuable help in editing the language of the manuscript.

References

[1] The British Pharmacopoeia 2012, The Stationary Office (TSO), Health Ministry, London, 2012.
[2] The United States Pharmacopoeia, 33 Reissue, The National Formulary 28, USP Convention, INC. Rockville, MD, 2010.
[3] F. Pena-Pereira, I. Lavilla, C. Bendicho, Trends Anal. Chem. 29 (2010) 617–628.
[4] I. Lavilla, F. Pena-pereira, S. Gill, M. Costas, C. Bendicho, Anal. Chim. Acta 647 (2009) 112–116.
[5] F. Pena-Pereira, I. Lavilla, C. Bendicho, Anal. Chim. Acta 631 (2009) 223–228.
[6] A.K.K.V. Pillai, A. Jain, K.K. Verma, Talanta 80 (2010) 1816–1822.
[7] F. Pena-Pereira, S. Senra-Ferreiro, I. Lavilla, C. Bendicho, Talanta 81 (2010) 625–629.
[8] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650–2656.
[9] A. Saleh, E. Larsson, Y. Yamini, J.Å. Jönsson, J. Chromatogr. A 1218 (2011) 1331–1339.
[10] Z. Es'haghi, R. Azmoodeh, Arabian J. Chem. 3 (2010) 21–26.
[11] A. Sarafraz-Yazdi, F. Mofazzeli, Z. Es'haghi, Chromatographia 67 (2008) 49–53.
[12] A. Sarafraz-Yazdi, A.H. Amiri, Z. Es'haghi, Talanta 78 (2009) 936–941.

[13] M.R.K. Zanjani, Y. Yamini, S. Shariati, J.A. Jonsson, Anal. Chim. Acta 585 (2007) 286–293.
[14] Z.Q. Ding, G.D. Liu, J. Chin., Anal. Chem. 37 (2009) 119–122.
[15] M. Zeeb, M.R. Ganjali, P. Norouzi, Microchim. Acta 168 (2010) 317–324.
[16] L. Kocúrová, I.S. Balogh, J. Škrlíková, J. Posta, V. Andruch, Talanta 82 (2010) 1958–1964.
[17] L. Rusnáková, V. Andruch, I.S. Balogh, J. Škrlíková, Talanta 85 (2011) 541–545.
[18] V. Andruch, L. Kocúrová, I.S. Balogh, J. Škrlíková, Microchem. J. 102 (2012) 1–10.
[19] A. Sarafraz-Yazdi, A. Amiri, Trends Anal. Chem. 29 (2010) 1–14.
[20] Validation of analytical procedures: text and methodology, ICH Harmonised Tripartite Guideline Q2(R1), November 1996.
[21] A.S. Douglas, M.W. Donald, Principles of Instrumental Analysis, Holt, Rinhart and Winston, New York, 1971, pp. 104.
[22] Product Safety Assessment: *n*-Butyl Acetate. Available from: 〈http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_01aa/0901b803801aa36e.pdf?filepath=productsafety/pdfs/noreg/233-00414.pdf&fromPage=GetDoc〉.