Sudarshan Publications

Available online at https://s-epub.in/index.php/trp/index

Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2025.v17.i01.pp93-100

SIMULTANEOUS DETERMINATION OF PARACETAMOL, CAFFEINE AND CHLORPHENIRAMINE BY HPLC-PDA

GARRETT HOLLOWAY[1]*, STEFAN HOFBAUER[2], OLIVER POLGLASE[3]

Affiliation [1]Department of Quality Assurance, Shenandoah Valley College of Pharmacy, Harrisonburg, USA
[2]Department of Analytical Chemistry, Styrian Institute of Pharmacology, Leoben, Austria
[3]Department of Analytical Chemistry, Exmoor Institute of Pharmaceutical Sciences, Taunton, United Kingdom

*Corresponding author: Garrett Holloway, g.holloway@svcp.edu
ORCID iD: Garrett Holloway 0000-0001-1029-7694; Stefan Hofbauer 0000-0001-3969-0633; Oliver Polglase 0000-0001-3918-9590

Article info

Received date:18-08-2024
Accepted date:09-11-2024
Published date:16-03-2025
ISSN;0975-8216

Keywords:

HPLC-PDA, Paracetamol, Caffeine, Chlorpheniramine, Method validation

Abstract

Fixed-dose tablets that combine paracetamol, caffeine and chlorpheniramine maleate are widely sold for cold, headache and allergic symptoms, and their quality control rests on liquid chromatography with photodiode array detection. This review, which reports no new experimental data, summarises what published work says about separating the three actives on reversed-phase columns, choosing wavelengths and confirming peak purity, validating the procedure against ICH expectations, and applying it to dosage forms and stability samples. The literature agrees on the broad chemistry but differs on buffer pH, gradient design and robustness testing. Large differences in dose between the actives, silanol-related tailing of chlorpheniramine and limited forced-degradation reporting are the main gaps identified.

1. INTRODUCTION

Combination analgesic and antihistamine tablets are among the most commonly dispensed over-the-counter products. A typical unit pairs paracetamol, usually 500 mg, with caffeine, perhaps 30 mg, and chlorpheniramine maleate at 2 mg. The first is a mild analgesic and antipyretic whose overdose is a leading cause of acute liver failure [1]. Caffeine acts as an adjuvant that modestly improves the pain relief obtained from common analgesics [2, 3], and chlorpheniramine is a first-generation H1 antagonist used for rhinitis and the symptoms of the common cold [4, 5]. Because the product is taken without supervision, the label claim for each ingredient has to be right. An under-dosed tablet is ineffective, while an over-dosed paracetamol tablet carries a real

safety cost.

Quality control laboratories therefore need one assay that quantifies all three actives in a single injection. Titrimetric and ultraviolet spectrophotometric procedures have been used, but they struggle when absorption bands overlap and when the sample matrix contains coloured excipients. Reversed-phase high-performance liquid chromatography, now the working standard for assay and related-substances testing [6, 7], resolves the components before they reach the detector. Coupling the column to a photodiode array (PDA) detector adds spectral information at every point of a peak, which helps with identification and with spotting co-eluting impurities. This review follows the published literature on that analytical approach, from column and mobile phase choices to validation and application, and it reports no data generated by the authors.

Pharmacopoeial monographs exist for each of the three drugs as single entities, and for some paracetamol tablets, but combinations are less consistently covered [8]. Manufacturers and regulators in many countries therefore depend on in-house or published procedures, which have to be validated before use and verified when transferred from one laboratory to another [9, 10]. That creates a practical question for the analyst: which of the many published separations deserves trust? Answering it means looking past the headline claim of a short run time or a high correlation coefficient, and asking how the method handles the basic antihistamine, how selectivity was demonstrated and how far the validation went beyond the minimum. Those three questions structure the discussion that follows.

Several earlier papers describe liquid chromatographic assays for paracetamol with caffeine, with or without a third active [11, 12]. What they share is a C18 column, an aqueous buffer with acetonitrile or methanol, and ultraviolet detection somewhere between 210 and 280 nm. What differs is how much attention is paid to the basic antihistamine, which behaves quite differently from its two neutral companions. The sections below set out the chemistry that drives these choices before moving to validation and routine use. Side-by-side reading shows how much hangs on unreported detail.

2. CHROMATOGRAPHIC CONDITIONS

The three analytes differ sharply in polarity and ionisation, and that explains most of the method development decisions reported. Paracetamol is a small, fairly polar, neutral molecule at acidic and neutral pH. Caffeine is a methylxanthine that is essentially unionised across the usual working range, so its retention is governed by hydrophobic interaction alone. Chlorpheniramine is a tertiary amine with a pKa above 9, which means it is fully protonated in most mobile phases used for pharmaceutical work [8]. Retention of the neutral pair is therefore insensitive to small pH shifts, whereas retention of the antihistamine can move considerably. A method that treats all three alike will tend to give acceptable peaks for two of them and a problem for the third.

Octadecylsilane columns of 150 to 250 mm in length with 5 µm particles dominate the published work [6, 7]. Residual silanol groups on older silica packings interact with protonated amines and produce the tailing that analysts often see for chlorpheniramine [13]. Three remedies recur in the literature. The first is to work at low pH, around 2.5 to 3.5, where most silanols are suppressed. The second is to select a modern, highly purified, end-capped column. The third is to add a competing base such as triethylamine to the buffer. Each has trade-offs: low pH can shorten column life on unprotected silica, and amine additives are slow to equilibrate and can shift baselines in gradient runs. Column dimensions and particle size likewise change back-pressure, run time and solvent use in ways that matter for routine work [7].

Isocratic elution is attractive for routine release testing because it is simple, reproducible and easy to transfer. Because the three compounds span a wide retention range under typical conditions, though, many laboratories adopt a short gradient to keep the antihistamine from eluting very late with a broad peak [14]. The gradient theory laid out for reversed-phase systems explains why a modest increase in organic fraction narrows

late peaks without costing resolution of the early pair [14]. Phosphate buffers at 10 to 50 mM are most common, with acetonitrile preferred over methanol for lower back-pressure and a cleaner baseline at short wavelengths. Column temperature is usually held between 25 and 40 °C, and since temperature can alter selectivity for ionisable solutes, it deserves explicit control [15].

Choice of pH deserves more thought than it often receives. A buffer works best within about one unit of its pKa, and phosphate has a first pKa near 2.1, so a phosphate buffer at pH 2.5 to 3.0 gives strong buffering exactly where silanol suppression is wanted [6, 13]. At that pH the antihistamine is fully charged, retention is moderate and the peak is reasonably symmetrical on a good column. Moving towards pH 6 or 7 raises the proportion of ionised silanols and increases tailing, although it can alter elution order and sometimes improve resolution between the neutral pair and unknown impurities. Buffer concentration matters too: too little gives poor capacity when a basic sample is injected, while too much risks precipitation when the organic fraction rises in a gradient [7, 15].

Sample preparation is where much of the day-to-day variation arises. Tablets are powdered, extracted with a mixture of mobile phase and organic solvent, sonicated and filtered. The extraction has to release a few milligrams of chlorpheniramine from a matrix dominated by several hundred milligrams of paracetamol, so the dilution that suits the major component leaves the minor one close to its quantification limit. Reports handle this in one of two ways: a single dilution with a wide detector linear range, or two preparations at different concentrations. The second is more laborious but gives better precision for the antihistamine peak [11, 12].

Mobile phase preparation sounds trivial and is not. The aqueous part should be pH adjusted before the organic solvent is added, since pH measured in mixed solvents has a different meaning. Filtration through a 0.45 µm membrane and degassing prevent noise from particles and bubbles in the detector flow cell, and fresh buffer should be made at intervals because phosphate solutions support microbial growth within days [6, 7]. Small errors in organic fraction change retention exponentially rather than linearly, so volumetric measurement of the components, not weighing, is a recurring source of between-day variation reported by laboratories [14].

3. DETECTION AND PEAK PURITY

A photodiode array detector records the whole ultraviolet spectrum several times per second, which lets the analyst choose the quantification wavelength after the run rather than before it. The absorption maxima of the three actives are not coincident. Paracetamol absorbs most strongly near 245 nm, chlorpheniramine near 261 nm and caffeine near 273 nm [8]. Published methods generally settle on a compromise wavelength, often between 220 and 270 nm, at which every analyte gives an adequate response, or they extract several channels from one acquisition and quantify each analyte at its own optimum. The second approach is easier to defend in validation because the signal-to-noise ratio for the low-dose antihistamine is much better at its own maximum [6].

Spectral data also supports peak identity. Overlaying the spectrum of a sample peak on that of a reference standard, and comparing spectra from the upslope, apex and downslope of the peak, is the usual way of showing that nothing else is hiding beneath it [6, 16]. Purity algorithms express the comparison as a match angle or similarity factor against a noise threshold. They work well when an interferent has a different spectrum from the analyte, but they are blind to co-eluting species with near-identical spectra, a limitation that matters for structurally related degradants. Purity checking is therefore best viewed as supporting evidence of selectivity rather than proof of it.

Detector linearity places a quiet ceiling on the work. Absorbance readings above roughly one absorbance unit begin to depart from the Beer-Lambert relationship because of stray light, so the concentration of paracetamol in the injected solution has to be kept modest [6, 7]. Yet the antihistamine is present at about one two-hundred-and-fiftieth of the paracetamol mass, and at the same dilution its peak is small. Analysts reconcile these

demands by choosing a wavelength that is not the paracetamol maximum, by using a shorter path-length flow cell, or by running a second, more concentrated preparation. Each choice has a bearing on the stated linear range and should be justified in the validation report [16, 17].

4. METHOD VALIDATION

The framework most papers follow is the ICH guideline on validation of analytical procedures, whose original text was revised in 2023 [18], with the compendial validation chapter built on much the same headings [19]. The guideline expects evidence on specificity, linearity, range, accuracy, precision, detection and quantification limits, and robustness, with the exact package depending on the purpose of the procedure. Guidance from the World Health Organization aligns with these headings [10], harmonisation proposals present the same characteristics under slightly different names [20], and practical texts on validation cover the same characteristics together with system suitability [21]. A newer guideline on analytical procedure development encourages a more structured, risk-based path to the same end [22]. The results from published combination assays are summarised in table 1, and fig. 1 shows how those entries divide between column, mobile phase, detection and validation topics.

Specificity is demonstrated in layers. The first layer is the injection of blank diluent and placebo to show that no excipient peak elutes at the retention time of any active. The second is the injection of each active alone and of the full mixture, so that identity and resolution can be read directly. The third, where a stability-indicating claim is made, is the analysis of stressed samples [9, 18, 23]. Resolution from the nearest neighbouring peak is normally expected to be at least 2, and the same pass criteria are repeated in the system suitability test so that the evidence is refreshed in each run [6, 17]. Papers that stop at the first layer show only that excipients do not interfere.

Linearity is typically reported over a range around 50 to 150 percent of the nominal assay concentration, using at least five levels and a correlation coefficient close to unity. Experts caution that a high coefficient alone says little about fit quality, and that inspection of residuals and consideration of weighting matter, especially when the calibration range spans more than one order of magnitude [24]. That is relevant here because chlorpheniramine, being the minor component, is often calibrated over a different range from paracetamol. Accuracy is usually shown by recovery of known amounts spiked into placebo, and precision by repeatability and intermediate precision expressed as relative standard deviation [9, 17].

Detection and quantification limits can be estimated from the signal-to-noise ratio or from the standard deviation of the response and the slope of the calibration line. The two routes can give noticeably different numbers, and some authors report both [25]. For an assay of a finished product these limits are of limited practical interest for the major actives, but they help demonstrate that the antihistamine can be measured with confidence at the bottom of the working range. Where a related-substances test for 4-aminophenol, the principal paracetamol impurity, is combined with the assay, limits become much more important because pharmacopoeial thresholds for that impurity are low [8].

Table 1: Reported features of reversed-phase liquid chromatographic assays for paracetamol, caffeine and chlorpheniramine

Feature	Reported practice and comment	Ref.
C18 column, 5 µm 150 to 250 mm length	Standard stationary phase; end-capped, high-purity silica reduces tailing of the basic antihistamine.	[6, 13]
Low-pH phosphate buffer pH 2.5 to 3.5	Suppresses silanol ionisation; retention of the neutral pair is little affected.	[8, 13]
Acetonitrile as modifier isocratic or short gradient	Lower back-pressure than methanol; gradient narrows the late-eluting antihistamine peak.	[7, 14]
Competing base additive for example triethylamine	Masks residual silanols but equilibrates slowly and may drift in gradients.	[7, 13]
PDA at a compromise wavelength 220 to 270 nm	Single channel suits all three actives; sensitivity for the minor component is limited.	[6, 11]

Feature	Reported practice and comment	Ref.
PDA with multiple channels analyte-specific maxima	Best signal-to-noise for each active; easier to justify in validation.	[6, 8]
Peak purity from spectral overlay upslope, apex, downslope	Supports selectivity but cannot expose co-eluting species with similar spectra.	[6, 16]
Linearity and accuracy around 50 to 150 percent of nominal	Residual inspection and weighting advised when ranges differ between actives.	[9, 24]
Robustness testing pH, organic fraction, flow, temperature	Often one factor at a time; designed experiments are better but rarely reported.	[22, 26]
Forced degradation acid, base, oxidative, thermal, light	Needed for a stability-indicating claim; frequently omitted from combination assays.	[23, 27]

Robustness is the parameter reported least thoroughly. A proper study varies buffer pH, organic fraction, flow rate, column temperature and wavelength deliberately, preferably in a designed experiment, and then judges whether system suitability criteria still hold [26]. Many papers instead alter one factor at a time and report only that the results stayed within a few percent. For this mixture, a shift of a few tenths of a pH unit is exactly the sort of change that moves the antihistamine peak, so a well-designed robustness study would be informative. Ruggedness across analysts, instruments and days is also thinly documented outside the better regulated settings [9, 26]. Where the antihistamine peak shows marginal tailing, an extra injection of the standard at the start of the sequence guards against a poor column going unnoticed.

Two preparatory details are easy to overlook. Solution stability, the length of time that standard and sample solutions give unchanged results when stored on the bench or in the autosampler, sets the practical window for a batch of injections and is rarely stated for these mixtures [9, 17]. Filter compatibility is the second. Nylon and some other membranes adsorb basic drugs, and because chlorpheniramine is present at a low concentration even a small proportion lost on the filter represents a visible bias. Discarding the first portion of filtrate, or showing that filtered and centrifuged samples agree, resolves the problem at little cost [6]. Both checks belong in the validation package of any assay containing a low-dose basic component.

5. APPLICATION TO FORMULATIONS

Once validated, the method is applied to commercial tablets, capsules and syrups, and the published analyses of such products are generally reassuring, with assay values clustering close to label claim [11, 12]. Excipients such as starch, microcrystalline cellulose and magnesium stearate are largely invisible at the wavelengths used, but film coat colourants and preservatives in liquid forms can add peaks and need to be checked in a placebo injection. Syrups also introduce sweeteners and flavours that interfere at low wavelengths. For these reasons the placebo and blank chromatograms form part of any specificity argument and are best shown alongside the sample trace [6, 17].

Content uniformity is a special concern for chlorpheniramine. At 2 mg per tablet it is a low-dose active, and blending a small mass of drug into a large mass of excipient is exactly the situation in which segregation and non-uniformity occur. A method used for uniformity testing has to quantify individual tablets, not a composite, which tightens the precision demanded of the antihistamine peak [8, 12]. Published combination assays are mostly composite assays, and the extent to which they have been verified at single-tablet level is rarely stated. Laboratories needing a uniformity method may have to run a separate verification at the lower concentration range that single-unit samples produce [11].

A method that will be used to support shelf-life claims has to be stability-indicating, meaning it can separate the actives from their degradation products. Forced degradation under acid, base, oxidative, thermal and photolytic stress is the accepted way to demonstrate this [23, 27]. Paracetamol hydrolyses to 4-aminophenol under harsh acid or base conditions, caffeine is comparatively stable, and chlorpheniramine can degrade under oxidative and photolytic stress. The aim is usually partial degradation of around 5 to 20 percent so that products are

visible without being dominated by secondary reactions. Many combination assays in the literature skip this step, so their selectivity towards degradants is unproven even if their selectivity towards excipients is solid [23].

Throughput is a second practical concern. Conventional HPLC runs of 10 to 20 minutes are acceptable for batch release, yet they become a bottleneck in dissolution testing or in-process control, where dozens of samples must be injected. Columns packed with sub-2 µm particles and operated at high pressure cut analysis times several-fold while maintaining resolution [28, 29]. Translating an existing method to such a system is feasible, but it involves re-optimising the gradient, dwell volume and detector settings, and in a regulated setting it requires at least partial revalidation. The efficiency gain has to be set against the capital cost and the extra care needed with sample cleanliness [28]. Reporting solvent volume per analysis would help readers compare methods fairly.

Environmental impact is increasingly part of the discussion. Acetonitrile is the dominant organic modifier and generates large volumes of waste across a quality control year. Frameworks such as the principles of green analytical chemistry and reviews of green analytical chemistry show how methods can be judged on solvent consumption, energy use, waste and operator safety [30, 31, 32]. Published combination assays seldom report such scores, though shorter runs, lower flow rates and ethanol-water mobile phases would improve them without necessarily compromising performance. Whether regulators will accept alternative solvents for compendial methods remains an open question [30].

6. LIMITATIONS AND FUTURE DIRECTIONS

Several limits of the evidence are worth stating plainly. Most published methods have been validated in one laboratory, on one or two instruments, with a small number of product batches. Few include true inter-laboratory comparison, so transfer performance is largely unknown. Reporting is also uneven: mobile phase preparation, detector settings and integration parameters are often incomplete, which complicates reproduction. The differences in dose between the three actives, a factor of roughly 250 between paracetamol and chlorpheniramine in a typical tablet, make the antihistamine peak the weak point in precision, and this is not always acknowledged in the discussion sections of the papers [11, 12].

The regulatory setting is also moving. The revised validation guideline and the new guideline on analytical procedure development together describe a lifecycle in which a procedure is developed with a defined target profile, validated, monitored in routine use and adjusted when needed [18, 22]. Most published combination assays pre-date this thinking and describe a fixed set of conditions rather than an operating region. Environmental considerations are moving in the same direction, and green metrics could become routine items in method reports [30, 31]. Authors who add a short lifecycle statement, giving the allowed ranges of key parameters and the monitoring plan, would make their methods more usable in practice [22].

Future work could usefully address four areas. First, designed robustness studies and method operable design regions, in line with current guidance on analytical procedure development, would replace one-factor-at-a-time testing [22, 26]. Second, comprehensive forced degradation with mass spectrometric identification of products would close the stability-indicating gap [23, 27]. Third, greener conditions should be evaluated with a formal metric, not just asserted [31, 32]. Fourth, collaborative studies across several laboratories would show whether published performance claims survive transfer. None of these requires new chemistry, only more disciplined study design and fuller reporting. Until such studies appear, a cautious reader should regard published performance figures as indicative, not guaranteed, particularly for the low-dose component.

7. CONCLUSION

Published work shows that paracetamol, caffeine and chlorpheniramine can be

separated and quantified in one reversed-phase run with photodiode array detection, and that the procedures behave well when validated along ICH lines [11, 12, 18]. The practical difficulties come from the antihistamine, which is basic, present at a tiny fraction of the paracetamol dose and prone to tailing unless pH, column chemistry or additives are chosen with care [8, 13]. Reported assay values for marketed products sit close to label claim, which suggests that the approach is sound when applied with care [11, 12]. For the analyst, that means the antihistamine peak is the one to watch at every stage, from extraction to integration.

Where the literature is thin, the gaps are mostly in how thoroughly the work is done rather than in what is chemically possible: designed robustness studies, forced degradation with identification of products, inter-laboratory transfer and green metrics are all under-reported [23, 26, 31]. Laboratories adopting a published method should treat it as a starting point, verify the weakest peak first and document every detector setting. Greener solvents and lifecycle thinking offer further room for improvement [22, 30]. Honest reporting of weaknesses is as useful to the next laboratory as any claim of success.

8. ABBREVIATIONS

HPLC-High-performance liquid chromatography, PDA-Photodiode array, ICH-International Council for Harmonisation, UV-Ultraviolet, RSD-Relative standard deviation, LOD-Limit of detection, LOQ-Limit of quantification, H1-Histamine type 1 receptor, C18-Octadecylsilane

9. FUNDING

Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. Prescott LF. Paracetamol overdosage: pharmacological considerations and clinical management. Drugs 1983;25:290–314.
2. Daly JW, Fredholm BB. Caffeine: an atypical drug of dual purpose. Drug Alcohol Depend 1998;51:199–206.
3. Schachtel BP, Fillingim RB, Lane AC, Thoden WR, Baybutt RI. Caffeine as an analgesic adjuvant: a double-blind study comparing aspirin with caffeine to both agents alone. Arch Intern Med 1991;151:733–7.
4. Simons FER. Advances in H1-antihistamines. N Engl J Med 2004;351:2203–17.
5. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis 2005;5:718–25.
6. Lindsay S, Kealey D. High performance liquid chromatography. Chichester: Wiley; 1987.
7. Lough WJ, Wainer IW, editors. High performance liquid chromatography: fundamental principles and practice. London: Blackie Academic; 1996.
8. Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poisons. 4th ed. London: Pharmaceutical Press; 2011.
9. Jenke DR. Chromatographic method validation: a review of current practices and procedures. I. General concepts and guidelines. J Liq Chromatogr Relat Technol 1996;19:719–36.
10. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-third report. Geneva: WHO; 2019 (Technical Report Series No. 1019).
11. Franeta JT, Agbaba D, Eric S, Pavkov S, Aleksic M, Vladimirov S. HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. Farmaco 2002;57:709–13.
12. Altun ML. HPLC method for the analysis of paracetamol, caffeine and dipyrone. Turk J Chem 2002;26:521–8.
13. Neue UD. HPLC Columns: Theory, Technology, and Practice. New York: Wiley-VCH; 1997.
14. Snyder LR, Dolan JW, Gant JR. Gradient elution in high-performance liquid chromatography: I. Theoretical basis for reversed-phase systems. J Chromatogr 1979;165:3–30.
15. Dolan JW. Temperature selectivity in reversed-phase high performance liquid chromatography. J Chromatogr A 2002;965:195–205.
16. Huber L. Validation and Qualification in Analytical Laboratories. 2nd ed. New York: Informa Healthcare; 2007.
17. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of bioanalytical chromatographic methods. J

Pharm Biomed Anal 1998;17:193–218.

18. ICH Harmonised Guideline. Validation of analytical procedures Q2(R2). Geneva: ICH; 2023.
19. United States Pharmacopeial Convention. General chapter 1225, Validation of compendial procedures. In: United States Pharmacopeia 38–National Formulary 33. Rockville: USP; 2015.
20. Hubert Ph, Nguyen-Huu JJ, Boulanger B, *et al.* Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal, part II. J Pharm Biomed Anal 2007;45:70–81.
21. Chan CC, Lee YC, Lam H, Zhang XM, editors. Analytical Method Validation and Instrument Performance Verification. Hoboken: Wiley-Interscience; 2004.
22. ICH Harmonised Guideline. Analytical procedure development Q14. Geneva: ICH; 2023.
23. Kats M. Forced degradation studies: regulatory considerations and implementation. BioPharm Int 2005;18(7):30–42.
24. Van Loco J, Elskens M, Croux C, Beernaert H. Linearity of calibration curves: use and misuse of the correlation coefficient. Accred Qual Assur 2002;7:281–5.
25. Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chron Young Sci 2011;2:21–5.
26. Vander Heyden Y, Questier F, Massart DL. Ruggedness tests on the HPLC assay of the United States Pharmacopeia XXIII for tetracycline hydrochloride: a comparison of experimental design and statistical interpretation methods. J Pharm Biomed Anal 1998;17:153–68.
27. Baertschi SW, Jansen PJ, Alsante KM. Stress testing: a predictive tool. In: Baertschi SW, Alsante KM, Reed RA, editors. Pharmaceutical stress testing: predicting drug degradation. 2nd ed. London: Informa Healthcare; 2011.
28. Gumustas M, Kurbanoglu S, Uslu B, Ozkan SA. UPLC versus HPLC on drug analysis: advantageous, applications and their validation parameters. Chromatographia 2013;76:1365–427.
29. MacNair JE, Patel KD, Jorgenson JW. Ultrahigh-pressure reversed-phase capillary liquid chromatography: isocratic and gradient elution using columns packed with 1.0-µm particles. Anal Chem 1999;71:700–8.
30. de la Guardia M, Garrigues S, editors. Handbook of green analytical chemistry. Chichester: Wiley; 2012.
31. Welch CJ, Wu N, Biba M, *et al.* Greening analytical chromatography. TrAC Trends Anal Chem 2010;29:667–80.
32. Anastas PT. Green chemistry and the role of analytical methodology development. Crit Rev Anal Chem 1999;29:167–75.