



*Dedicated to Professor Ionel Haiduc
on the occasion of his 75th anniversary*

SYNTHESIS AND HPLC SEPARATION OF NEW QUINUCLIDINE DERIVATIVES

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Six new optically active esters have been synthesized by the esterification of benzoic, salicylic or acetylsalicylic acid chlorides with truncated cinchona alkaloids quincorine and quincoridine respectively. Structural assignments were based on high resolution ¹H-NMR and IR spectroscopy. A HPLC method for the quantification of quincorine acetylsalicylate was optimized and validated.

INTRODUCTION

During the last decades, the tremendous development of asymmetric synthesis was highly supported by powerful organic chiral auxiliaries. The readily available and less expensive cinchona alkaloids (*e.g.* quinine, quinidine, cinchonine, cinchonidine), offering chiral skeletons and multiple possibilities of supplementary functionalization, were often selected for diverse types of asymmetric reactions.¹ The characteristic quinuclidine structural unit, contains 4 chiral centres and a nitrogen atom imprinting alkaline properties, thus making the cinchona alkaloids suitable ligands for a variety of metal-catalyzed processes.

Some representative examples are the osmium-catalyzed asymmetric dihydroxylation of olefins,² enantioselective heterogeneous asymmetric hydrogenation of α -keto esters and asymmetric syntheses using anionic nucleophiles under phase-transfer conditions.³

Quinidine and quinine alkaloids have been transformed into quincoridine (QCD) **1** and

quincorine (QCI) **2** (Fig. 1), by the cleavage of the C₄-C₉ bond in the corresponding cinchona alkaloids. Each of these two optically active 2-(hydroxymethyl)-5-vinylquinuclidine stereoisomers preserve intact the four chiral centres and thus, are considered as “truncated” alkaloids. The enantiomerically pure quinuclidine derivatives proved to be convenient intermediates for drug syntheses as well as ligands or catalysts in mediated asymmetric syntheses, similar to their parent alkaloids.^{4,5}

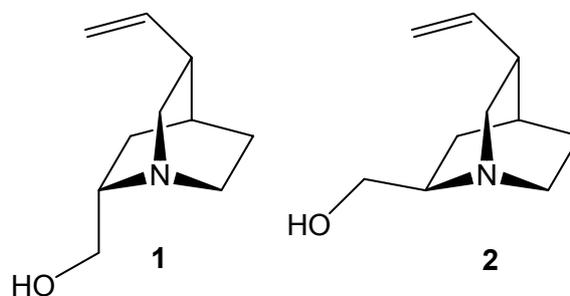
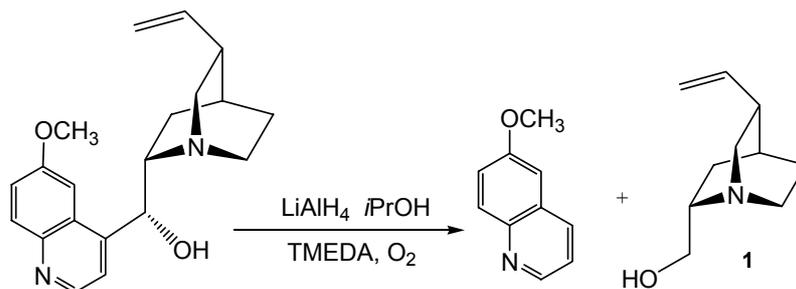


Fig. 1 – Structural formulae of “truncated” alkaloids: quincorine (QCI) **1** and quincoridine (QCD) **2**.

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Scheme 1

The successful cleavage of quinine, into 6-methoxy-quinoline and the corresponding enantiomerically pure 2-(hydroxymethyl)-1-azabicyclo[2.2.2]octane (quincorine QCI) **1**, is shown in Scheme 1.⁵

Quinine was transformed into ester derivatives either by transesterification (*e.g.* salicylquinine prepared starting with salol), or by the reaction with acyl halides (*e.g.* the reaction with pyridine dicarboxylic acid halide⁶).

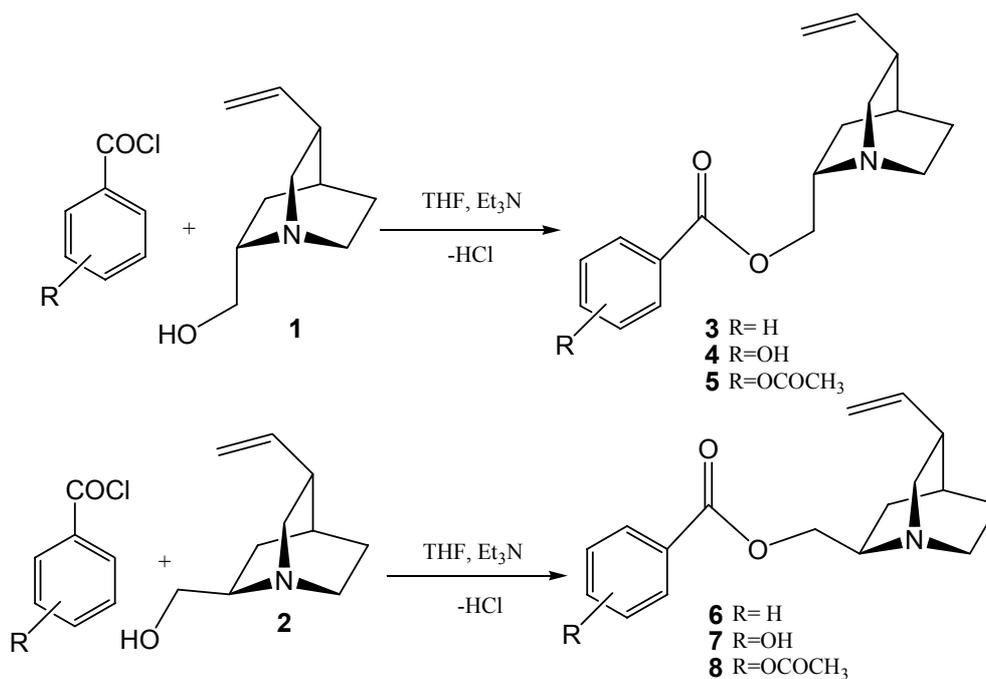
Several methods for the determination of cinchona alkaloids either in drug composites, in body fluids or in pharmaceutical formulations, were described.⁷⁻¹² Chromatographic techniques, especially HPLC, have played the most important role in facilitating the detection and quantification of these compounds. The techniques employed included HPLC,⁷⁻⁹ GC¹⁰ and spectrophotometric methods.^{11,12}

The aim of this work was the preparation of new esters containing the optically active quinuclidine

unit, starting with truncate cinchona alkaloids (QCI) **1** and (QCD) **2** and salicylic acid derivatives. The new esters may be considered as building blocks with huge potential in the development of new and effective medicinal compounds. A search for HPLC optimal conditions for the quantification of quincorine acetylsalicylate (QCI-ASA), was conducted in order to develop a rapid, accurate, reproducible and valid method for the determination these new esters.

RESULTS AND DISCUSSION

The truncated cinchona alkaloids QCI **1** and QCD **2** were used as alcohol precursors for new benzoates, salicylates and acetylsalicylates respectively containing the chiral quinuclidine core, as shown in Scheme 2.



Scheme 2

The quincorine esters **3-5** and quincoridine **6-8** esters were obtained in good yields when the corresponding truncated cinchona alkaloids were treated with an aromatic acyl halide in the presence of triethylamine (Et₃N). Benzoates **3,6** were obtained in 84% yields, but in the case of salicylates **4,7** and acetylsalicylates **5,8**, the reaction yields decreased to 73% and 55% respectively, due to the steric hindrance induced by the bulky substituent in the *ortho* position of the aromatic ring.

The new esters were characterized by high resolution ¹H-NMR, IR spectroscopy and elemental analysis. The presence of the ester group was confirmed in the IR spectra of **3-8**, by the characteristic absorption bands situated at 1710-1750 cm⁻¹ (stretching vibrations of C=O), 1238 and 1165 cm⁻¹ (stretching vibrations of C-O). ¹H-NMR spectra confirmed the presence of the quinuclidine unit with preserved configurations at each of the 4 chiral centres.

The HPLC conditions were adjusted in order to provide a good assay performance in the separation of quincorine acetylsalicylate (QCI-ASA). Columns Nucleosil C18, Nucleosil C8, Hipersil C18 and Lichrosphere RP18 were successively tested; Nucleosil C18 and Hipersil C18 gave comparable results. A variety of mobile phases were investigated in the development of an HPLC method suitable for analysis of the new esters. These included methanol-water 70:30 (% v/v), acetonitrile-water 70:30 (% v/v), methanol-phosphate buffer (pH=3) 50:50 (% v/v). The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, suitability for stability studies, time required for the analysis.

The objective of method validation is to demonstrate that the method is suitable for the intended purpose as stated in ICH guidelines.¹³ The method was validated for linearity, precision, accuracy specificity, short-term stability and system suitability. Standard plots were constructed with four concentrations in the range 10-50 µg/mL. The peak area signal was plotted against the corresponding concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from five replicate injection 50µg/mL of the intended test

concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area was determined and precision was reported as % R.S.D. Sample solution short-term stability was tested at ambient temperature (20 ± 3 °C) for three days. In order to confirm the stability of standard solution, protected from light were reinjected after 24 and 48h at ambient temperature and compared with freshly prepared solution.¹⁴

The optimum mobile phase containing acetonitrile and phosphate buffer (pH 3) 50:50 v/v with 0.03 mol/l hexylamine, was selected because it could resolve the peak (RT = 9.57 min) with a resolution factor of 13.14, capacity 1955 and asymmetry factor 1.10. Quantification was achieved with UV detection at 254 nm on the basis of peak area at 0,5 mL/min flow rate. A typical HPLC chromatogram obtained during determination QCI – ASA is given in Fig. 2.

Five different concentrations (10, 25, 40 and 50 µg/mL) were prepared for linearity studies. The calibration curve obtained by plotting area against concentration showed a linear relationship over a concentration range of 10-50 µg/mL. The linear regression equations were found to be $y=879166x-4854$. The regression coefficient value ($R^2=0.9978$) indicating a high degree of linearity. Calibration curves are shown in Fig. 3.

Regression characteristics of the proposed HPLC method are given in Table 1.

Precision

Standard solutions were injected using a universal Rheodyne injector of 20 µL. The intra-day and inter-day precision were assessed by analyzing standard solution. The %RSD was found to be between 0.47 and 0.67. The lower values of % RSD indicate that the method is precise.

System suitability

This test was performed by collection of data from replicated injection of QCI-ASA solution given in Table 3. Standard deviation of retention times and of the peak areas were 9.57 and 86958, respectively. The mean theoretical plates count was 18203 (Table 2).

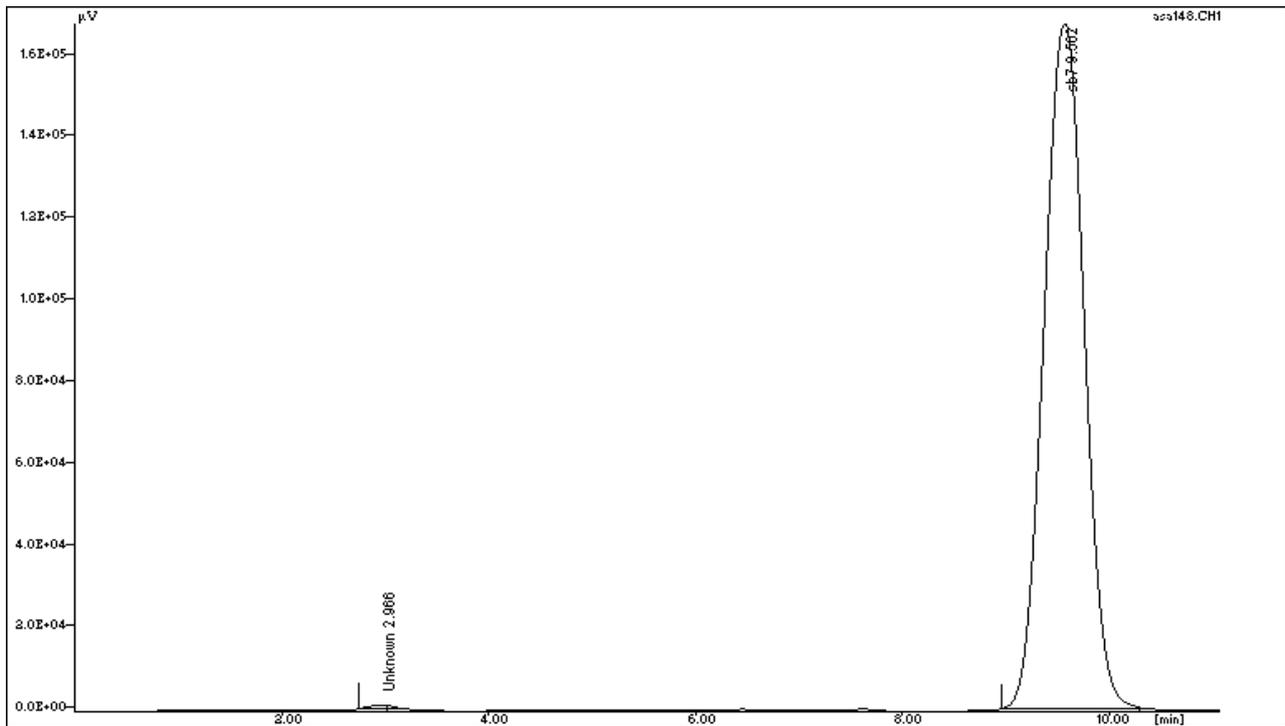


Fig. 2 – HPLC chromatogram of QCI-ASA, column: Nucleosil C18, eluent: acetonitrile:phosphate buffer (pH 3) 50:50 v/v with 0.03 mol/L hexylamine, 0.5 mL/min flow rate.

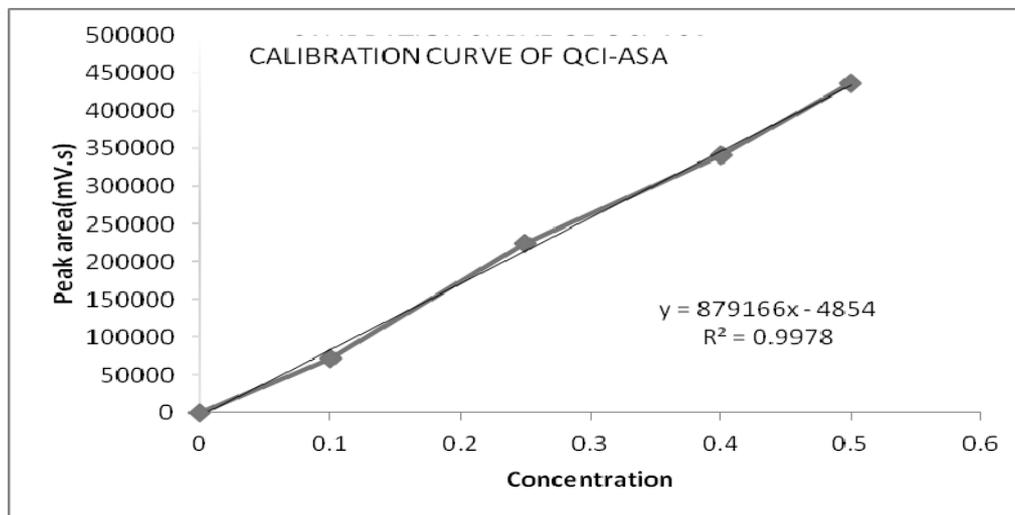


Fig. 3 – Calibration curve of QCI-ASA.

Table 1

Regression characteristics of the proposed HPLC method

Linearity experiment	
Range ($\mu\text{g/mL}$)	10 -50
Regression coefficient (R^2)	0.9978
Slope	879166
Intercept	4854

Table 2

System suitability data

Injection number	Retention times(min)	Peak area (mV·s)	Plates	Asymmetry
1	9.58	87377	18157	1.12
2	9.56	89432	18034	1.11
3	9.60	90368	18034	1.13
4	9.55	90143	18122	0.98
5	9.54	86421	18111	1.21
6	9.56	81437	18263	1.04
7	9.59	88573	18106	1.09
8	9.57	83548	18077	1.12
9	9.59	85321	18157	1.12
Means	9,57	86958	18203	1.10
RSD(%)	0.21	1.35	1.41	0.41

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification were found to be 0.01 µg/mL and 0.035 µg/mL. The values indicate that the method is sensitive. Studies of the effects of exhaustive stress conditions, separation and identification of the degradation products, are currently in progress in our laboratory.

EXPERIMENTAL

General. Preparative column chromatography was performed on J.T. Baker silica gel (particle size 30-60 mm), analytical TLC was carried out on aluminium-backed 0,2 mm silica gel 60 F₂₅₄ plates (E. Merck). ¹H-NMR spectra were recorded on a Bruker AM 400 spectrometer in CDCl₃ unless otherwise stated. The HPLC were performed on a Able&Jasco chromatographic system equipped with a pump module PU-1580, ternary gradient module LG-980-02S, degasser module DG-980-50, with a manual injector Rheodyne 7125 valve fitted a 20 µl volume sample loop, equipped with a UV detector UV-1575 and a Nucleosil C18, 5µm, 250 x 4 mm column. All solution were filtered through 0,45 mm Millipore filter prior.

HPLC Sample preparation: QCI-ASA 25 mg accurately weighted and transferred to a 25 mL volumetric flask and to this 10 mL Methanol was added and then diluted with methanol to 1mg/mL. A volume of each solution was diluted to 25 mL with methanol to get final the concentrations of 50, 40, 25, 10 µg/mL. Each resulting solution was filtered through 0,45 µm pore size filter units.

Reagents QCI, QCD were purchased from Innochem GmbH, Et₃N, hexylamine, KH₂PO₄, methanol HPLC grade and acetonitrile HPLC grade from Merck.

Salicyloyl chloride and acetylsalicyloyl chloride were prepared by general method of synthesis from acids and SOCl₂.¹⁵

General procedure for Esterification of truncate cinchona alkaloids:

To a well-stirred mixture of acyl chloride (1.1 eq) in THF and Et₃N (0.3 mL/0.001 mol QCI/QCD) was added one of QCI or QCD (1 eq). Stirring was continued for 3h at reflux and then, aqueous NaHCO₃ was added followed by an excess

of dichloromethane. The organic phase was collected and washed with water (3×20 mL). The organic phase was dried over anhydrous Na₂SO₄, the dichloromethane removed under vacuum and the resulting oil collected. The prepared compounds were purified using column chromatography on silica gel.

(1S,2S,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl benzoate 3

(η=84%) ¹H-RMN:(400 MHz, CDCl₃): δ (ppm) 8.08 (m, H-14,H-18); 7.42-7.36(m,H-15, H-16,H-17); 4.48-4.43(dd, J=4.4, 11.3 H-9); 4.28(dd, J=6.3, 10.6 H10); 3.49-3.46(m, H-2); 3.42-3.36(m, H-8); 3.22-3.06(m,H-7); 2.81-2.76(ddd, J=3.8 8.1 11.8 H-6); 2.02-1.93(m, H-4); 1.74(d,J=6.5 H11); 1.49-1.42(m, H-3).C₁₇H₂₁NO₂(272,16): calc. C 75.02%, H 8.07%, N 5.14%; det. C 74.77%, H 7.66%, N 8.32%.

(1S,2R,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl

benzoate 6 (η=84%). ¹H-RMN:(400 MHz, CDCl₃): δ 8.12(m, H-14,H-18); 7.95-7.87(m,H-16.);7.49(m, H-15,H-17); 4.57-4.51(dd, J=8.6,12.3 H-9); 4.16(dd, J=6.1, 12.3 H10); 3.73-3.62(m, H-7); 3.58-3.52(dd,J=5.8 14.1 H-6); 3.39-3.26(m,H-2); 3.18(m,H-5);3.06-3.01(ddd, J=2.6 8.9 14.4 H-6); 2.52(m, H-7); 2.23-2.17(m, H-4, H-8, H-3); 1.77-1.74(d, J-6.5 H-11). C₁₇H₂₁NO₂(272.16): calc. C 75.02%, H 8.07%, N 5.14%; det. C 74.61%, H 7.52%, N 8.47%.

(1S,2R,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl salicylate 4

(η=79%) ¹H-RMN: (400 MHz, CDCl₃): δ ppm 7.95(m, H-14); 7.22-7.28(m,H-15, H-16.);6.94-6.91(m, H-17); 5.70-5.63(dd, J=6.1 10.6 H10); 4.47-4.43(dd, J=4.9, 11.6 H-9); 3.11-3.08(m, H-2); 2.84(m, H-8); 2.73(m,H-7); 2.67-2.64(ddd, J=3.9 8.1 11.5 H-6); 2.11(m, H-5); 1.96(m, H-4); 1.73-1.69(d,J=6.4 H11); 1.58(m, H-3).C₁₇H₂₁NO₃(288.36): calc. C 70.83%, H 7.64%, N 4.86%; det. C 69.23%, H 7.86%, N4.57%.

(1S,2S,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl salicylate 7

(η=73%) ¹H-RMN:(400 MHz, CDCl₃): δ ppm 7.82(m, H-14); 7.27(m,H-16);6.94(m,H-15, H-17); 4.87-4.78(dd, J=4.3, 11.6 H-9, HOH); 4.38(dd, J=6.4, 10.6 H10); 3.34(m, H-2); 3.11-3.09(m, H-7); 2.89(m,H-5); 2.73-2.68(ddd, J=3.9 8.1 12.1 H-6); 2.04(m, H-4); 1.49-1.44(d,J=6.5 H11, H-8); 1.67(m, H-3). C₁₇H₂₁NO₃(288.36): calc. C 70.83%, H 7.64%, N 4.86%; det. C 69.84%, H 7.75%, N4.63%.

(1S,2S,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl acetylsalicylate 5

(η=65 %) ¹H-RMN:(400 MHz, CDCl₃): δ ppm 7.93(m, H-14); 7.64(m,H-16.);7.29-7.23(m, H-15, H-16); 5.41-5.37(m,H-10,H-11);4.43-4.34(dd, J=3.8, 8.1 H-9); 3.38-3.42(m, H-2); 2.87-2.81(ddd, J=3.9 8,1 11.5 H-6); 2.45(m, H-7); 2.39(m, H-

5); 2.24-2.11(m, H-3, CH₃); 1.83-1.79(m, H-4). C₁₉H₂₄NO₄(330.42): calc. C 69.10%, H 7.27%, N 4.24%; det. C 68.36%, H 7.46%, N 4.22%.

(1S,2R,4S,5R)-5-vinyl-1-azabicyclo[2.2.2]oct-2-yl-methyl acetylsalicylate 8 (η=55%). ¹H-RMN:(400 MHz, CDCl₃): δ ppm 7.96(m, H-14); 7.44-7.36(m, H-16); 7.17-7.11(m, H-15, H-16); 5.72-5.69(dd, J=6.9, 11.6 H10); 5.03(m, H-11); 4.43-4.37(dd, J=4.1, 8.6 H-9); 3.27-3.18(ddd, J=3.9 8.1 11.5 H-6); 2.56-2.49(m, H-7); 2.27-2.21(m, H-3, CH₃); 1.52-1.47(m, H-4, H-8). C₁₉H₂₄NO₄(330.42): calc. C 69.10%, H 7.27%, N 4.24%; det. C 70.36%, H 7.16%, N 4.83%.

CONCLUSIONS

New esters of truncate cinchona alkaloids were obtained in good yields, based on the reaction of quincoridine or quinconine with benzoic, salicylic or acetylsalicylic acid chlorides. The structures of the new products were assigned based on ¹H-NMR, IR spectroscopy and elemental analysis.

The HPLC method elaborated for quantification of QCI-ASA is accurate, precise and reproducible. It is also characterized by a wide range of linearity, uses of an inexpensive and readily available mobile phase, UV detection and no internal standard.

REFERENCES

1. C. E. Song, "Cinchona Alkaloids in synthesis and catalysis", Wiley, 2009.
2. H. C. Kolb, M. S. Van Nieuwenhze and K. B. Sharpless, *Chem. Rev.*, **1994**, *94*, 2483.
3. T. Hashimoto and K. Maruoka, *Chem. Rev.*, **2007**, *107*, 5656.
4. H.M.R. Hoffmann and J. Frackepohl, *Eur. J. Org. Chem.*, **2004**, *21*, 4285-4315.
5. H.M.R. Hoffmann (Buchler GmbH Brunswick), *US Pat.* 5.777122, Jul.7, **1998**.
6. H. Cheng, Y. Jin and R. Jians, *Catalysis Communication*, **2008**, *9*, 1858-1862.
7. Z. Gong, Z. Zhang and X. Yang, *Analyst*, **1997**, *122*, 283-285.
8. D.V. McCalley, *J. Chromatography A*, **2002**, *967*, 1-19.
9. D.V. McCalley, *J. Chromatography A*, **1986**, *357*, 221-226.
10. R. Verpoorte and A.B. Svendsen, "Chromatography of Alkaloids, part B, Gas-liquid chromatography and high performance liquid chromatography", *Journal of Chromatography Library*, Elsevier, **1984**.
11. P. B. Marshall and E. W. Rogers, *Biochem J.*, **1945**, *39*, 258-262.
12. A.O. Gettler and I. Sunshine, *Anal. Chem.*, **1951**, *23*, 779-781.
13. EMEA-ICH Q2(R1), "Validation of Analytical Procedures", 1995.
14. L. Roman, M. Bojita, R. Sandulescu and D.L. Muntean, "Validarea metodelor analitice", Ed. Medicală, 2007.
15. H. Cousse and G. Mouzin (Pierre Fabre S.A.), *US Pat.* 4006181, Febr. 1, **1977**.