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Journal of Chromatography A, 853 (1999) 479–485

JOURNAL OF CHROMATOGRAPHY A

Capillary electrophoretic separation of *N*-acetylcysteine and its impurities as a method for quality control of pharmaceuticals

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Abstract

Capillary electrophoresis has been applied to separate and determine *N*-acetylcysteine (NAC) and related impurities. Determination conditions were found to be optimum with 100 mmol/l borate as the buffer, pH 8.40. The limit of detection was established for each substance examined. The method has been validated by examining linearity ranges, precision and repeatability. The method was used to determine the content of NAC in, and purity of, pharmaceutical preparations. The major impurities (*N,N*-diacetylcystine, *N,S*-diacetylcysteine and cystine) were determined at levels of 0.1%.

Keywords: Pharmaceutical analysis; Acetylcysteine; Cystine; Cysteine

1. Introduction

N-Acetylcysteine (NAC) is a mucolytic drug widely used in the treatment of respiratory diseases. Its sulfhydryl (–SH) group reacts with disulfide bonds in mucoproteins to split them into smaller units, whereby the viscosity of the mucus becomes reduced [1]. As a thiol containing molecule NAC possesses antioxidant properties, which are causing its importance in clinical applications to grow. It has been identified as a glutathione precursor and, thus, it can modulate thiol tissue levels and provide protection from hepatotoxic, nephrotoxic or another impairing agents [2–4]. NAC can enhance the capacity of the organism to detoxicate free radicals [5]. It has been applied to treat induced intoxications, e.g. by paraquat and paracetamol [6,7], and also to reduce the toxicity of cytostatic drugs like cyclophosphamide and cisplatine [4,8]. Because of its antioxidant properties NAC is considered to have a place as an antiviral [9,10], antineoplastic [11,12] and antiinflammatory agent [13], and it is also being proposed that it can be used in the virus therapy for acquired immunodeficiency syndrome (AIDS) and hepatitis B, for cancer prevention and in the treatment of oxidative stresses of various origins [14,15].

The quality control of NAC-containing drugs requires, among other things, the active substance to be determined and the purity of a preparation to be established by determining the level of impurities contained. Major NAC impurities include cysteine, cystine and *N,S*-diacetylcysteine, which are formed while NAC is being synthesized, and *N,N*-diacetylcystine, which is formed as a product of oxidation while NAC is being stored (Fig. 1, based on Refs. [16,17]).

An HPLC method for assaying NAC and by-products has been described in the European Pharmacopoeia [18]. The monograph on *N*-acetylcysteine for the Polish Pharmacopoeia V is already prepared. Therefore, resort was taken in capillary electropho-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00727-X

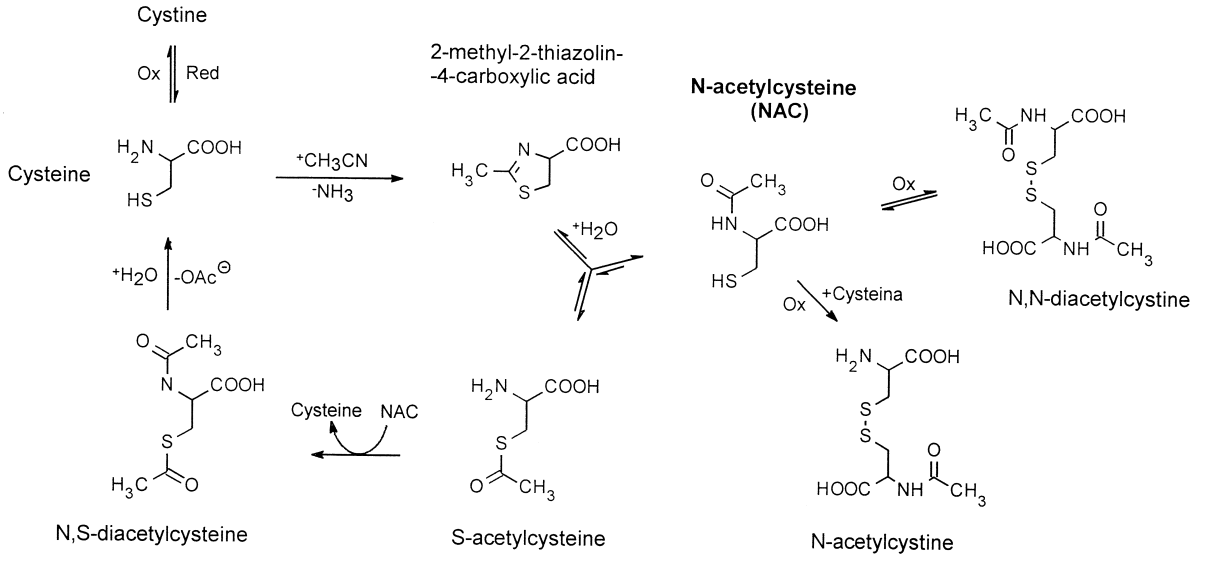

Fig. 1. The scheme of *N*-acetylcysteine transformations (based on Refs. [16,17]).

resis (CE) to determine the purity of NAC-containing preparations.

Capillary electrophoresis, a recent analytical technique (since 1981), using capillaries as a support medium, is considered as an alternative to HPLC. This technique uses the force of a strong electric field to separate charged compounds. As compared with chromatographic methods, CE represents a completely different separation technique, yet it offers levels of accuracy, precision and sensitivity similar to those achieved with HPLC.

2. Experimental

2.1. Materials and methods

Standard substances: L-cysteine, L-cystine and benzyl alcohol were purchased from Sigma; *N*-acetylcysteine, *N*,*S*-diacetylcysteine and *N*,*N*-diacetylcystine were purchased as European Pharmacopoeia standards from Promochem.

Commercial preparations containing *N*-acetylcysteine in various pharmaceutical forms (tablets, capsules, effervescent tablets, injections, granulates, syrups) were tested.

Each solution was prepared by using Baker's analytical reagent grade chemicals.

Capillary electrophoresis was carried out in a fused-silica capillary 60 cm (53 cm to detector)×75 cm μm I.D. by using a Quanta 4000 (Waters, USA) equipment. A constant voltage of 25 kV, with the resulting current intensity of about 30–40 μA, was applied. UV absorption was measured at 214 nm. Samples were introduced hydrodynamically with an elevation of 10 cm and the injection time was 10 or 15 s.

Prior to the separation, the capillary was flushed for 10 min with 0.1 mol/l NaOH and conditioned for another 10 min with the background electrolyte (BGE). During the analysis a 2-min purge with buffer was applied.

Separation was provided in the background electrolyte containing a borate buffer, 100 mmol/l, pH 8.40. Benzyl alcohol was used as an internal standard and an electroosmotic flow (EOF) marker.

3. Results

To optimize the method, the pH range 8.0–9.4 was examined at steps of 0.2. Peak migration times

were used to calculate the ionic mobility (μ_e) (Eq. (1)) for each substance investigated

$$\mu_e = \mu_a - \mu_{EOF} = \frac{L \times l}{V \times t_m} - \frac{L \times l}{V \times t_{EOF}} \quad (1)$$

where μ_e = ionic mobility, μ_a = apparent mobility, μ_{EOF} = electroosmotic flow mobility, L = total length of capillary, I = distance to detector, V = voltage applied, t_m = migration time, t_{EOF} = migration time of EOF marker.

In Fig. 2, the ionic mobility is plotted against the pH of the separating buffer. Buffers having a pH of 8.8 and more offered poor resolving power and protracted the analysis time. Within the pH range of 8.0–8.6 changes in ionic mobility were insignificant and with a pH of 8.4, the resolution of cystine and cysteine peaks attained maximum.

After optimization, and by using the conditions described above, all compounds were separated within less than 10 min (Fig. 3). The separation efficiency was very high, N was 350 000 (estimated from the peak width at half height). The high concentration of the buffer improved the resolution of cystine from cysteine.

The detection limits were established for each substance examined, viz., 75 μg/ml for cysteine and 5 μg/ml for cystine, *N*-acetylcysteine, *N*,*S*-diacetylcysteine and *N*,*N*-diacetylcystine (Fig. 4).

Peak area in relation to concentration was examined (linearity). Results are shown in Table 1. The validation of the present method was complemented by checking the repeatability within and between determinations. Results are listed in Table 2.

The method developed was used to determine *N*-acetylcysteine and related derivatives in various pharmaceutical preparations. Determination of impurities in pharmaceutical preparations requires NAC-containing specimens to be separated at high concentrations of NAC. The present method allows separation, identification and determination of all the compounds examined at NAC concentrations of 5–10 mg/ml. The permissible impurity concentrations in pharmaceuticals, combined with the detection limits, are presented in Table 3.

None of the preparations examined were found to

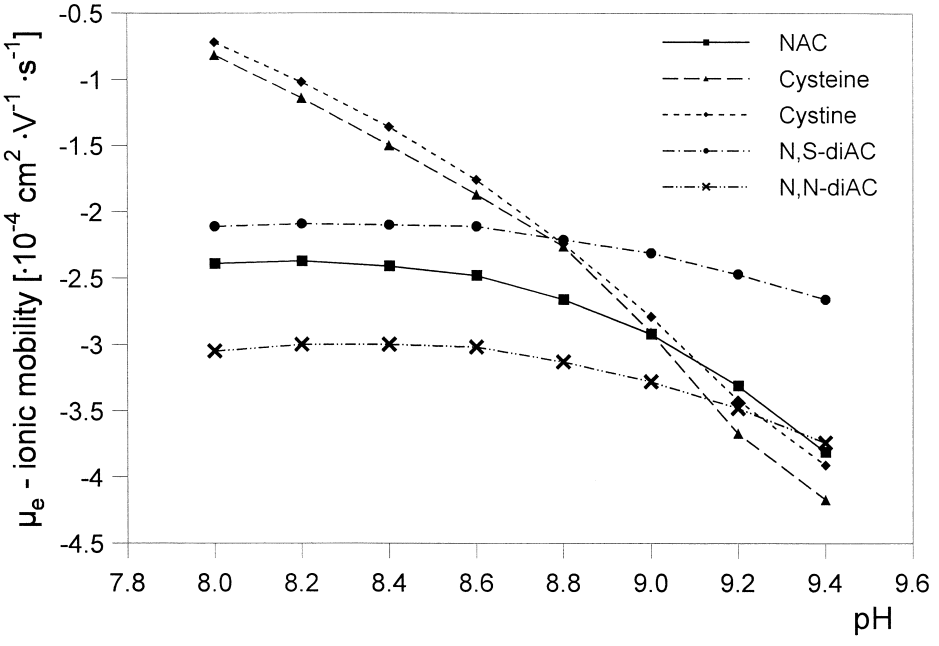

Fig. 2. Effect of pH on the ionic mobility (μ_e) each compound investigated. Plot shows the best separation conditions at pH 8.40. Electrophoresis was performed at conditions described in Section 2 (except for the pH of BGE). IS = Internal standard (benzyl alcohol); CYS = cysteine; NAC = *N*-acetylcysteine; *N*,*S*-diAC = *N*,*S*-diacetylcysteine; *N*,*N*-diAC = *N*,*N*-diacetylcystine.

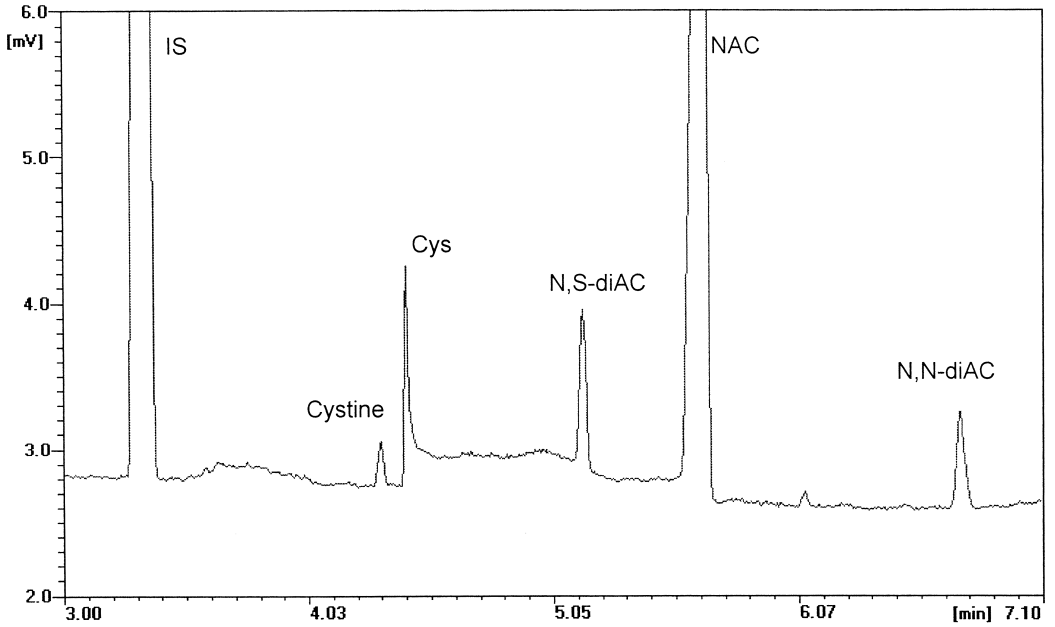

Fig. 3. Electrophoretic separation of standards of *N*-acetylcysteine and its impurities. Separation conditions: 60 cm (53 cm to detector)×75 μm I.D. fused-silica, 100 m*M* borate buffer, pH 8.40, voltage 25 kV, detection at 214 nm. Abbreviations as in Fig. 2.

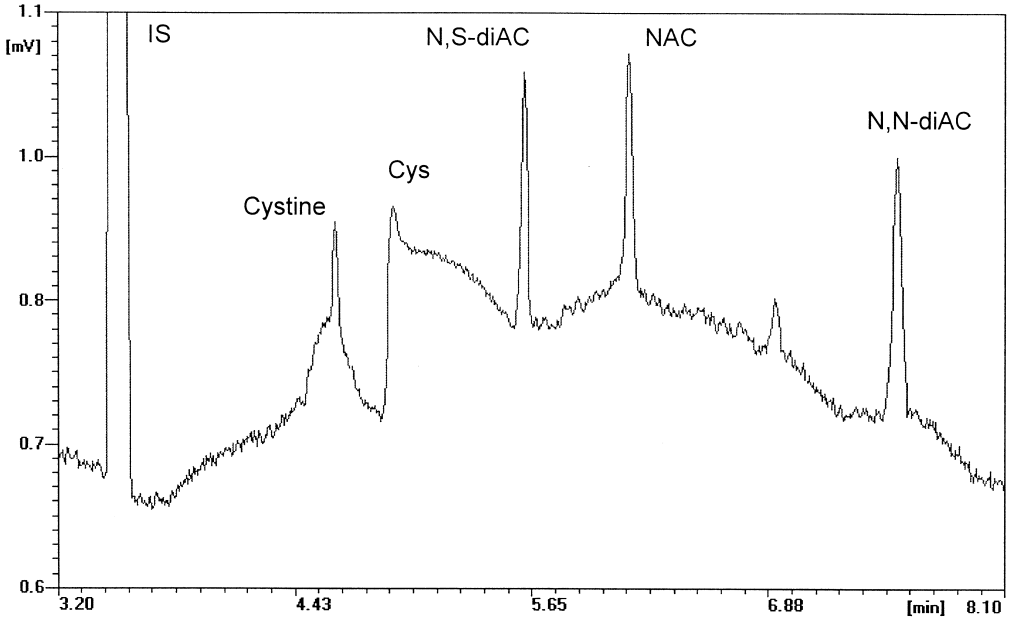

Fig. 4. Absolute detection limit of all compounds under investigation. Abbreviations as in Fig. 2. For concentrations see Section 3. Separation conditions as in Fig. 3.

Table 1
Linearity[a]

	Linearity range (μg/mol)	Correlation coefficient (*R*)	*N*[b]
N-Acetylcysteine	50–11 000	0.9999	12
Cystine	5–110	0.9992	10
Cysteine	100–1300	0.9995	6
N,*S*-Diacetylcysteine	10–1000	0.9999	10
N,*N*-Diacetylcystine	10–1000	0.9999	10

[a] Peak area in relation to concentration.
[b] *N* = Number of data points.

Table 2
Repeatability within and between determinations (*N* = 5)

	Repeatability, RSD (%)		Concentration range examined (mg/ml)
	Within tests	Between tests	
N-Acetylcysteine	0.42	1.24	0.50–3.00
Cystine	2.72	8.61	0.01–0.03
Cysteine	1.27	1.79	0.15–0.55
N,*S*-Diacetylcysteine	1.42	3.02	0.02–0.06
N,*N*-diacetylcystine	2.59	3.34	0.02–0.06

contain cystine or cysteine. In some preparations *N*,*S*-diacetylcysteine was detected. All the preparations examined were found to contain, in varying amounts, the product of the oxidation of *N*-acetylcysteine, viz., *N*,*N*-diacetylcystine. Pharmaceuticals with an expired shelf-life were found to contain more *N*,*N*-diacetylcystine and other unidentified peaks than those within their shelf-life.

During the electrophoresis, the carbohydrates used in the preparations as excipients were found not to interfere with the substances examined. *N*-acetylcysteine and its derivatives could also be determined in the preparations containing such auxiliaries as methyl *p*-hydroxybenzoate (Nipagin M, Methylparaben), propyl *p*-hydroxybenzoate (Nipagin P, Propylparaben) and saccharin. Representative electrophoregrams of the ACC Saft preparation and ACC 200 effervescent tablets are shown in Figs. 5 and 6, respectively.

4. Discussion

The present method for determining *N*-acetylcysteine, *N*,*S*-diacetylcysteine, *N*,*N*-diacetylcystine, cysteine and cystine was validated and found to lend itself to the quantitative determination of each component in a mixture. The resolution attained is satisfactory even in the cases of cystine and cysteine. The irregular peak of cysteine (strong

Table 3
The comparison of the permissible impurity concentration in pharmaceuticals (as a percentage of the main component)

	Absolute detection limit (μg/ml)	Purity level in pharmaceutical preparation (%)	Maximum permissible impurity concentration in 10 mg/ml of NAC solution (μg/ml)
Cystine	5	≤2.0	200
Cysteine	75	≤1.5–2.0	150–200
N,*S*-Diacetylcysteine	5	≤0.5	50
N,*N*-Diacetylcystine	5	≤0.5–1.0	50–100

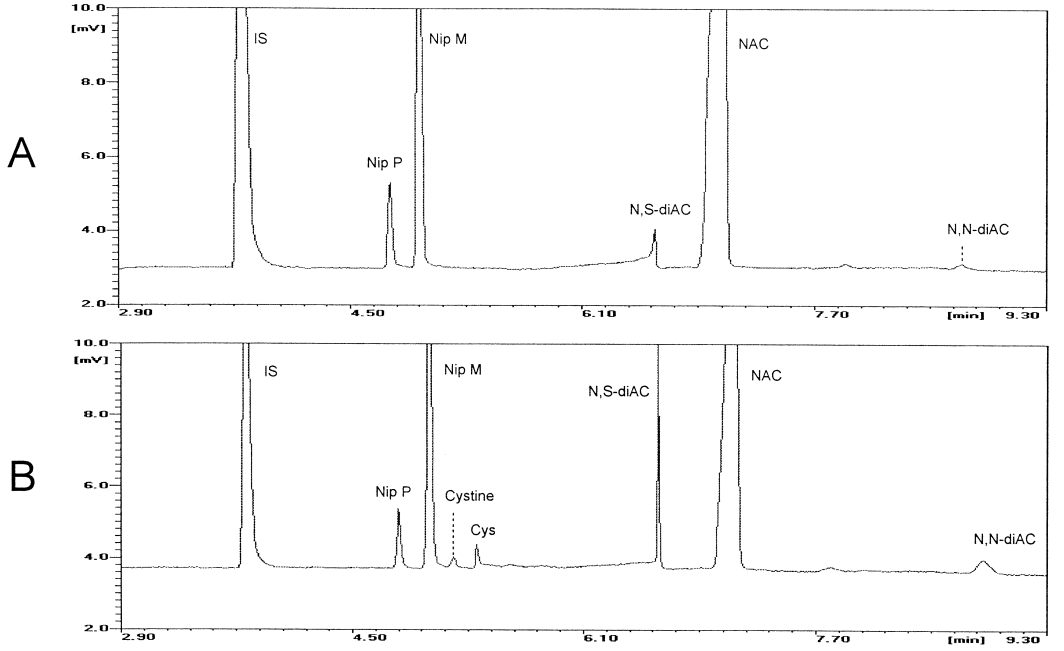

Fig. 5. (A) Electrophoregram of the ACC Saft preparation (a granulate to prepare a syrup); (B) electrophoregram of the above specimen fortified with impurity standards. Nip P = Propyl *p*-hydroxybenzoate (Propylparaben), Nip M = methyl *p*-hydroxybenzoate (Methylparaben), other abbreviations as in Fig. 2. Separation conditions as in Fig. 3.

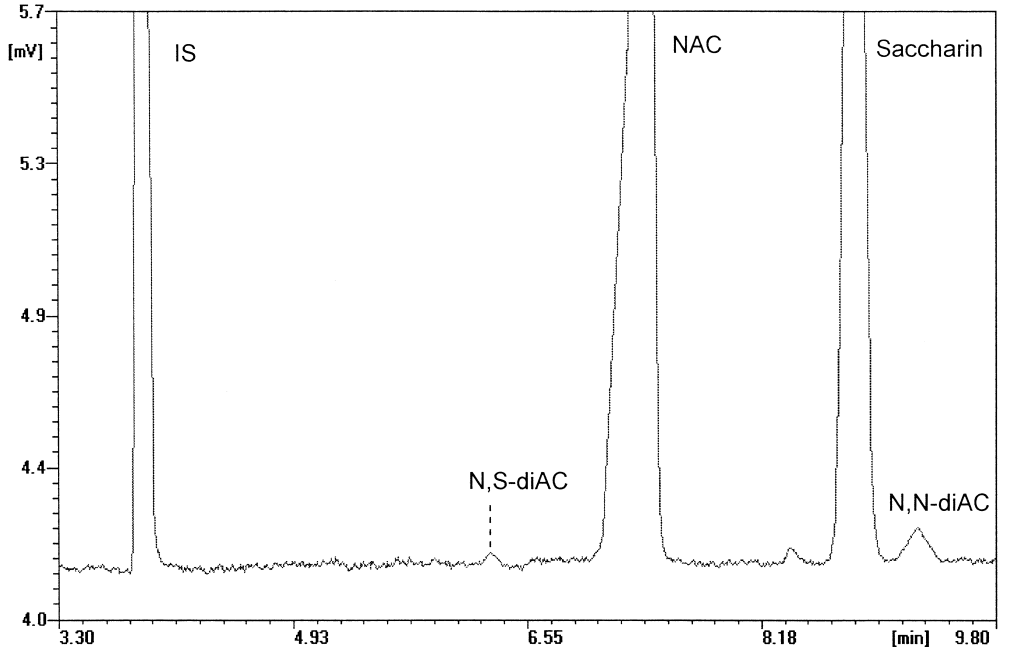

Fig. 6. The electrophoregram of ACC 200 effervescent tablets. Abbreviations as in Fig. 2. Separation conditions as in Fig. 3

tailing) does not allow the value of resolution to be calculated precisely, but signals between peaks reach the baseline so they are well distinguishable. It seems the same reason to induce a high detection limit of cysteine comparing with other analytes. However, the peak has been clearly identified and the signal significantly exceeded baseline noise.

The limits of detection of the individual substances are lower by a few magnitudes than the impurity concentrations permissible for the preparation solutions containing *N*-acetylcysteine at a concentration of 10 mg/ml (see Table 3).

The present method lends itself to studying *N*-acetylcysteine preparations provided in the form of syrups, granulates or effeverscent tablets, because excipients like saccharose, sorbitol, mannitol and lactose have no effect on the resolvability and determinability of the compounds investigated. Other auxiliaries like methyl *p*-hydroxybenzoate (Methylparaben), propyl *p*-hydroxybenzoate (Propylparaben) and saccharin do not interfere with the determination of *N*-acetylcysteine and its impurities, and they too can be identified and determined.

5. Conclusions

The method described allows the separation and determination *N*-acetylcysteine and related impurities as well as auxiliaries like Methylparaben, Propylparaben and saccharin.

The low detection limits, the broad linearity ranges and high repeatability make it possible to determine quantitatively the substances examined to a degree comparable with that attained by HPLC but within a shorter time and at a lower cost of analysis. The auxiliaries constituting the basis of the drug form, including saccharose, sorbitol, mannitol and lactose, affect neither the resolvability nor the determinability of the compounds investigated.

References

[1] R.P. Tomkiewicz, E.M. App, G.T. De Sanctis, M. Coffiner, P. Maes, B.K. Rubin, M. King, Pulm. Pharm. 8 (1995) 259.
[2] A. Pastor, P.S. Collado, M. Almar, J. Gonzales-Gallego, J. Hepat. 27 (1997) 363.
[3] J.C. Thies, T.A. Koeppel, T. Lehmann, P. Schemmer, G. Otto, S. Post, Transpl. Proc. 29 (1997) 1326.
[4] D. Sheikh-Hamad, K. Timminis, Z. Jalali, J. Amer. Sci. Nephrol. 8 (10) (1997) 1640.
[5] L. van der Laan, W.J. Oyen, A.A. Verhofstad, E.C. Tan, H.J. ter Laak, A. Gabreels-Festen, T. Hendriks, R.J. Goris, J. Surg. Res. 72 (1997) 60.
[6] A.L. Jones, D.R. Jarvie, D. Simpson, P.C. Hayes, L.F. Prescott, Aliment. Pharm. Therap. 11 (1997) 787.
[7] C.A. Bowden, E.P. Krenzelok, Drug Safety 16 (1997) 9.
[8] E. Hoeffer, I. Avidor et al., Toxicol. Appl. Pharmacol. 120 (1993) 8.
[9] L. Weiss, E. Hildt, P.H. Hofschneider, Antiviral Res. 32 (1996) 43.
[10] B. Akerlund, C. Jarstrand, B. Lindeke, A. Sonnerborg, A.C. Akerblad, O. Rasool, Eur. J. Clin. Pharm. 50 (1996) 457.
[11] Y. Delneste, P. Jeannin, L. Potier, P. Romero, J.Y. Bonnefoy, Blood 90 (1997) 1124.
[12] S. De Flora, C.F. Cesarone, R.M. Balansky, A. Albini, F. D'Agostini, C. Bennicelli, M. Bagnasco, A. Camoirano, L. Scatolini, A. Rovida et al., J. Cell. Biochem. Suppl. 22 (1995) 33.
[13] U. Savla, P.H. Sporn, C.M. Waters, Am. J. Physiol. 237 (1997) L1013.
[14] K.L. Brigham, Eur. Respir. J. 3 (1990) 482–484.
[15] H.F. Galley, P.D. Howdle, B.E. Walker, N.R. Webster, Free Radical Biol. Med. 23 (1997) 768.
[16] H. Wätzig, C. Dette, A. Aigner, L. Wilschowitz, Pharmazie 49 (1994) 249.
[17] H.A. Smith, G. Gorin, J. Org. Chem. 26 (1961) 820.
[18] Monograph on acetylcysteine, in: European Pharmacopoeia, 3rd ed., European Department for the Quality of Medicines within the Council of Europe, Strasbourg, 1997, p. 343.