Available online at www.sciencedirect.com
SCIENCE DIRECT®
JOURNAL OF CHROMATOGRAPHY A
Journal of Chromatography A, 1070 (2005) 155–161
www.elsevier.com/locate/chroma

# Development of a solid-phase extraction/gas chromatographic–mass spectrometric method for quantification of succinic acid in nucleoside derivatives for oligonucleotide synthesis

Åke Stenholm [a,*], Ingrid Drevin [b], Michael Lundgren [c]

[a] *Process Analytical Chemistry, Amersham Biosciences, Björkgatan 30, S-75184 Uppsala, Sweden*
[b] *Analytical Chemistry, Chemical Characterization, R&D, Amersham Biosciences, Björkgatan 30, S-75184 Uppsala, Sweden*
[c] *Analyscentrum, Casco Products AB, Box 11538, S-10061 Stockholm, Sweden*

Received 30 November 2004; received in revised form 7 February 2005; accepted 18 February 2005
Available online 13 March 2005

**Abstract**

A solid-phase extraction (SPE)/gas chromatographic–mass spectrometric (GC–MS) method was developed for analysing residual succinic acid in nucleoside derivatives to be used in oligonucleotide synthesis. Use of a SPE protocol, enabled most of the derivatives to be trapped, thereby creating eluates enriched in succinic acid. GC–MS was used to quantify the amount of residual succinic acid in four different nucleoside preparations, with succinate concentrations varying from 0.18 to 0.24% (w/w). The within-day repeatability of the method was found to be 1.25% RSD. A linear relationship was observed between the amount of succinic acid in the sample and the GC–MS peak area, with a correlation coefficient of 0.9997 in the concentration interval 0.05–2.5% (w/w). Recoveries were measured by the addition of internal standards to working solutions and varied between 99.8 and 102.6%.
© 2005 Elsevier B.V. All rights reserved.

*Keywords:* Succinic acid; Nucleoside derivatives; Solid-phase extraction protocol

## 1. Introduction

The quantitative analysis of succinic acid in nucleoside derivatives to be used in oligonucleotide synthesis is important since the activation step preceding coupling of the derivatives to amino-derivatised supports can be affected by the presence of monomeric succinic acid. The aim of this study was to develop a method for quantifying succinic acid in nucleoside derivatives. Gas chromatography–mass spectrometry (GC–MS) has proved to be a sensitive tool for analysing the trimethylsilyl derivative of succinic acid [1–4]. SPE is commonly used for sample isolation and preconcentration [5–7]. In this study, isolation was necessary since preliminary GC–MS results showed that high injector temperatures lead to cleavage of the 4,4′-dimethoxytrityl group of the nucleosides, resulting in unclear chromatograms. The literature suggests that temperatures above 195 °C should be avoided in nucleotide analyses [8–10]. In this study, the removal of nucleoside derivatives prior to GC–MS analysis made it possible to use elevated injector temperatures. As well as being heat-sensitive, the 4,4′-dimethoxytrityl group is acid-labile [11]. Fig. 1 shows the chemical structure of the investigated nucleoside derivatives and the 4,4′-dimethoxytrityl side group, respectively.

The choice of a suitable SPE stationary phase should be guided by the chemical characteristics of the sample. Commercially available phases can be classified as non-polar, polar or ion-exchange [7]. The purpose of the part of this study concerned with SPE was to identify a stationary phase that would enable efficient elution of succinic acid and trapping of the nucleosides. Lindström et al. [12] tested three non-polar phases ($C_8$, $C_{18}$, $C_{18}$EC) and one polar phase (ENV+) for extracting succinic acid from an aqueous solution containing

* Corresponding author. Tel.: +46 18 6121224; fax: +46 18 6121847.
*E-mail address:* ake.stenholm@amersham.com (Å. Stenholm).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.02.071

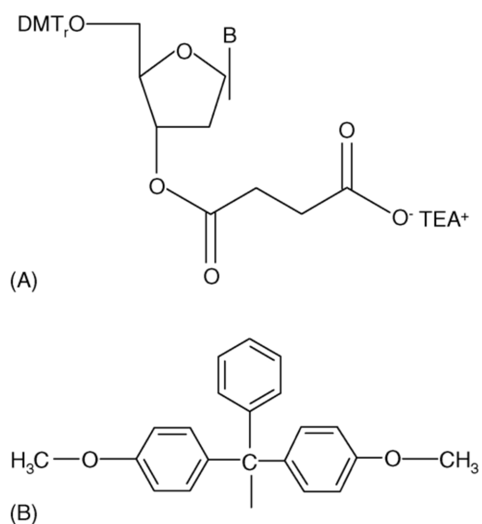


Fig. 1. (A) Structures of nucleoside derivatives. 5′-*O*-DMTr-$N^6$-benzoyl-2′-deoxyadenosine-3′-*O*-succinate, TEA salt (nucleoside adenosine derivative) and 5′-*O*-DMTr-thymidine-3′-*O*-succinate, TEA salt (nucleoside thymine derivative). B = bases, adenosine (A) and thymine (T), TEA = triethylamine, DMTr = 4,4′-dimethoxytrityl. (B) Structure of the 4,4′-dimethoxytrityl group.

hydrolysates of poly (butylene adipate) and poly(butylene succinate). de Villiers et al. [13] extracted succinic acid from wine adjusted to pH 2.5 by use of styrene-divinylbenzene (SDB) cartridges. The use of anion-exchange sorbents to extract organic acids has been described by various authors. Glowniak et al. [6] used quaternary amine sorbents for extracting phenolic acids from plant materials at a pH, 2 units higher than the acid's p$K_a$. Suárez-Luque et al. [14] removed organic acids including succinic acid from honey using anion-exchange techniques. Ng et al. [3] used strong anion exchange (SAX) disks for extracting organic acids (including succinic acid) from distilled alcoholic beverages. The use of a combined $C_{18}$/anion-exchange technique for cleaning balsam vinegars prior to GC-analysis is described by Cocchi et al. [4]. However, the use of anion exchangers would have been impractical in this study as the nucleosides were in ionic form. One advantage of performing extractions with non-polar sorbents at neutral pH is that it simplifies sample handling [15].

## 2. Experimental

### 2.1. Chemicals

Succinic acid (>99.5%) and ethyl acetate, analytical-reagent grade were purchased from Merck, Darmstadt, Germany. [1,2,3,4-$^{13}C_4$] succinic acid (99%) was obtained from Cambridge Isotope Las., Andover, MA, USA. BSTFA [*N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide] was purchased from Pierce, Rockford, IC, USA. HPLC grade acetonitrile was obtained from Fisher Scientific, Leicestershire, UK. Water was of Milli-Q gradient grade, Millipore, Bedford MA, USA. 5′-*O*-DMTr-$N^6$-benzoyl-2′-deoxyadenosine-3′-*O*-succinate, TEA salt (nucleoside adenosine derivative) and 5′-*O*-DMTr-thymidine-3′-*O*-succinate, TEA salt (nucleoside thymidine derivative) were purchased from Pierce Milwaukee, WI, USA.

### 2.2. Choice of SPE sorbent

The nucleoside derivatives are hereafter denoted ND, NAD (nucleoside adenosine derivative) and NTD (nucleoside thymine derivative). The capacities of three SPE sorbents were tested for their capability to extract nucleoside derivatives for oligonucleotide synthesis from succinic acid in an acetonitrile/water solution. Isolute ENV+ (6 ml, 200 mg), Isolute MFC18 (3 ml, 200 mg) and Isolute 101 (6 ml, 200 mg) all from Sorbent, Västra Frölunda, Sweden, were the sorbents of choice. ENV+ is a cross-linked hydroxylated polystyrene-divinylbenzene copolymer designed for extracting polar compounds [16]. ENV+ was tested in our study, mainly because the nucleoside derivatives were present as salts, and as such could possibly have bound more strongly to the stationary phase than succinic acid. However, the non-polar nature of the 4,4-dimethoxytrityl group prompted us to also test two non-polar stationary phases: Isolute 101 and Isolute MFC18. Isolute 101 is a highly cross-linked polystyrene-divinylbenzene copolymer, while Isolute MFC18 is a non end-capped octadecyl sorbent [16]. Of these three stationary phases, Isolute 101 is the least and ENV+ the most polar sorbent. The nucleoside derivatives were initially dissolved in a mixture of acetonitrile and water and then subjected to SPE. Since silylation of the acids was considered necessary for the GC–MS analyses, the eluates were then freeze-dried to remove the water [17]. The pH of nucleoside derivatives dissolved in acetonitrile/water is approximately 7. Succinic acid has p$K_a$ values 4.16 and 5.61 [18], so succinic acid is thus ionized at pH 7 and does not bind to the non-polar sorbent. The solid phases were initially activated with 2 ml acetonitrile and conditioned with 2 ml water. Sample clean up was performed by diluting 0.5 ml of approximately 20 mg/ml nucleoside thymine derivative in acetonitrile with 4 ml of water. The solutions were then added to the sorbent columns. For the Isolute MFC18 sorbent, half the solution volume was added as the column reservoir was only 3 ml. Each of the columns was then rinsed with 1 ml water. The eluates from the three sorbents were tested for nucleosides, by applying 1-μl samples to Merck thin-layer chromatography (TLC) plates (aluminium sheets 20 cm × 20 cm, Silica gel 60 F254). The presence of NTDs was monitored by illumination with a Camag UV lamp at 254 nm. The TLC-spot originating from the Isolute 101 eluate was only slightly visible, showing that this sorbent trapped the nucleosides most efficiently.

### 2.3. Liquid chromatography–ultraviolet spectrophotometry (LC–UV)

LC–UV was used to determine the recovery of NTD in the development phase of the SPE protocol. The amount

of residual NTD in the Isolute 101 sorbent was analyzed with the Agilent (Palo Alto, CA, USA) 1100+Agilent DAD. A Thermo Hypersil 100 mm × 2.1 mm, 3 μm HiPurity $C_{18}$ column was used with a mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), starting with 30% B and rising over 25 min to 100% B. This concentration was maintained for 10 min.The column was then equilibrated and conditioned to 30% B for 5 min before the next injection. The injection volume was 3 μl and the wavelength selected for monitoring the eluate was 266 nm.

### 2.4. Freeze drying of SPE purified samples

The purified samples were transferred to 2-ml autosampler-vials. The vials were freeze-dried in a Christ Alpha 2-4 system (Martin Christ Gefriertrocknungsanlagen), at an operating pressure of 0.77 for approximately 12 h.

### 2.5. Gas chromatography–mass spectrometry (GC–MS)

One millilitre of acetonitrile and 100 μl of BSTFA was added to vials containing SPE-purified, freeze-dried samples. The vials were capped and heated in a heating block (QBT1 from Grant, Cambridgeshire, UK) at 70 °C for 15 min. The silylated derivatives were identified and quantified using an Agilent GC–MS system comprising an Agilent 6890 GC and an Agilent 5973 MS. The column used was a wall-coated open-tubular (WCOT) fused silica HP-5 MS column from Agilent (30 m × 0.25 mm i.d., df 1 μm). Helium of scientific grade purity from AGA (Stockholm, Sweden) was used as carrier gas at 1.5 ml/min. Electronic pressure control (EPC), of the GC run was used to control the flow velocity during each GC run. The initial oven temperature was 90 °C which was maintained for 1 min, before being raised to 300 °C at a rate of 15 °C/min and maintained at 300 °C for 1.5 min. A split/splitless injector with a splitless liner (Agilent 5062–3587) was used to perform the injections in splitless mode at 250 °C with an injection volume of 1 μl.

Ionization was performed with an electron energy of 70 eV. The transfer line temperature was set to 280 °C. The identification of succinic acid was confirmed by comparing sample retention times and mass spectra to the corresponding standard compound. Quantification was done using single ion monitoring (SIM) mode at *m*/*z* 247 (succinic acid) and *m*/*z* 251 ($^{13}$C succinic acid). The latter substance was used as internal standard (IS).

### 2.6. Preparation of standard solutions for linearity study

Hundred milligrams of succinic acid was dissolved in 10 ml tetrahydrofuran in a 50 ml measuring flask and diluted with 40 ml water (solution S1, 2000 μg/ml). A series of dilutions was performed in order to obtain standard solutions of different concentrations as shown in Table 1.

Table 1
Preparation of standard solutions for linearity study of succinic acid

| Dilution procedure | Solution | Concentration (Hg/ml) |
|---|---|---|
| 100 mg succinic acid + 10 ml tetrahydrofuran + 40 ml of water | S1 | 2000.0 |
| 1 ml of S1 + 7 ml of water | S2 | 250.0 |
| 3 ml of S2 + 6 ml of water | S3 | 83.3 |
| 3 ml of S3 + 6 ml of water | S4 | 27.8 |
| 3 ml of S4 + 6 ml of water | S5 | 9.3 |
| 3 ml of S5 + 6 ml of water | S6 | 3.1 |

The dilutions were performed either with an Eppendorf Multipipette or with ordinary glass pipettes. Calibration solutions were prepared by placing 1.00 ml of the standard solutions in a 10 ml glass vial and adding 3 ml of water, 0.5 ml of acetonitrile and 50 μl of IS solution (984 μg/ml $^{13}$C succinic acid in acetonitrile). The samples for the linearity study were not SPE-extracted but were otherwise treated in the same fashion as the ND containing samples—i.e. they were subjected to freeze-drying, derivatization and GC–MS analysis.

## 3. Results and discussion

### 3.1. Development of a solid-phase extraction protocol

The main goal was to develop a protocol that achieves extraction with high precision and good recovery.

Isolute 101, a non-selective and generally applicable organic polymer sorbent, was chosen as the most suitable of the three sorbents considered. The adsorption mechanism of this phase is based on dipole–dipole or dipole-induced-dipole interactions between neutral, non-polar, or weakly polar molecules and the sorbent. The extraction yield depends on the lipophilicity of the substances, which in the case of acidic/basic molecules, is dependent on the solution's pH [19]. The ability of the sorbent to extract acidic, basic and neutral substances has previously been investigated by Baselt and Cravey [20].

#### 3.1.1. Recovery of nucleoside derivatives

Two samples were prepared to determine the amount of residual NTD retained by the Isolute 101 sorbent. For this, 0.5 ml of 19.86 mg/ml NTD in acetonitrile was diluted with 4 ml water. The sample was added to an activated Isolute 101 column and then rinsed with 1 ml water (**1**). The reference sample was prepared by diluting a 0.5 ml 19.86 mg/ml NTD sample with 5 ml water (**2**). Fifty microlitres of (**1**) was added to 1 ml acetonitrile:water (1:1, v/v). Ten microlitres of (**2**) was added to 1 ml acetonitrile:water (1:1). From the HPLC analysis of sample (**1**), a peak with 60 area counts was recorded. From the analysis of (**2**), a peak with 1386 area counts was recorded. The conclusion is that the eluate contained less

Table 2
Recovery of succinic acid using Isolute 101, SPE columns

| Sample | Succinic acid (area counts) | IS (area counts) | Ratio succinic acid/IS | Average ratio | Recovery, (B/A average × 100) (%) |
|---|---|---|---|---|---|
| A1[a] | 85216 | 145689 | 0.5849 | | |
| A2 | 134721 | 230195 | 0.5852 | 0.5856 | |
| A3 | 142009 | 242074 | 0.5866 | | |
| B1[b] | 113859 | 197211 | 0.5773 | | 98.6 |
| B2 | 112403 | 194397 | 0.5782 | | 98.7 |
| B3 | 75966 | 131256 | 0.5788 | | 98.8 |

[a] Samples without SPE cleanup.
[b] Samples with SPE cleanup.

than 1% of NTD. Over 99% of NTD was thus retained by the sorbent.

NDs bind strongly to the sorbent, presumably due to the hydrophobic character of the 4,4′-dimethoxytrityl groups they contain. NTDs dissolve readily in acetonitrile. However, adding NTDs dissolved in pure acetonitrile to SPE cartridges leads to lower recovery of the derivatives. In the study, 0.5 ml of approximately 20 mg/ml NTD was diluted with 4 ml water. This volume was added to the Isolute 101 cartridge. A higher water:acetonitrile volume ratio leads to incomplete dissolution of the derivatives. The chosen water:acetonitrile ratio (8:1, v/v) was found to be optimal after testing other ratios with the simple TLC–UV illumination technique described in the Section 2.

### 3.1.2. Recovery of succinic acid

For quantification purposes, succinic acid should not be retained in the Isolute 101 sorbent. The recovery of the acid was tested by GC–MS analyses of succinic acid solutions without SPE cleanup (A) and with SPE cleanup (B). A-solutions consisted of 1.0 ml 27.7 μg/ml succinic acid in water + 4 ml water + 0.5 ml acetonitrile. Fifty microlitres of IS solution (984 μg/ml $^{13}$C succinic acid in acetonitrile) was added to A-solutions; B-solutions had 1.0 ml 27.7 μg/ml succinic acid in water + 3 ml water + 0.5 ml acetonitrile. The recovery experiments were performed in triplicate for A- and B-solutions. For B-solutions, prior to extraction, the columns were first activated with 2 ml acetonitrile and then conditioned with water. After extraction, the columns were rinsed with 1 ml water. Fifty microlitres of IS solution (984 μg/ml $^{13}$C succinic acid in acetonitrile) was added to B-solutions after cleanup. Approximately, 1 ml of each solution was transferred to separate 2 ml autosampler vials with screwcaps loosely screwed on. The vials were frozen in liquid nitrogen and put in a freeze drier over night to dry. The dried samples were derivatised with 100 μl of BSTFA dissolved in 1 ml acetonitrile. The vials were then capped and heated in a heating block at 70 °C for 15 min. Portions (0.5 μl) of the silylated samples were injected in the GC–MS system. Succinic acid was detected at 247 *m/z* and IS at 251 *m/z*. The conclusion from this recovery investigation is that less than 1.5% of the succinic acid solution added to an Isolute 101 column is retained. (Table 2).

Table 3
Effect of liquid–liquid extraction (LLE) with ethyl acetate on the recovery of NTD

| Sample | Area counts at 266 nm | Recovery (LLE/ref × 100) (%) |
|---|---|---|
| NTD sample 1 with LLE | 5.8 | 2.8 |
| NTD sample 1 without LLE (ref) | 206.8 | |
| NTD sample 2 with LLE | 4.1 | 4.4 |
| NTD sample 2 without LLE (ref) | 94.2 | |

### 3.1.3. Liquid–liquid extraction step

In order to remove the residual ND that was not trapped in the sorbent, a liquid–liquid extraction step for the eluates was introduced. Andrews et al. [21] used butanol for extracting nucleosides from a DNA-hydrolysate. The presence of the 4,4′-dimethoxytrityl group in ND suggested the use of a less polar solvent; ethyl acetate was chosen. Four samples from the same NTD batch were dissolved and added to Isolute 101 columns using the procedure described in the Section 2. Two of the eluates were vigorously extracted with 4 ml ethyl

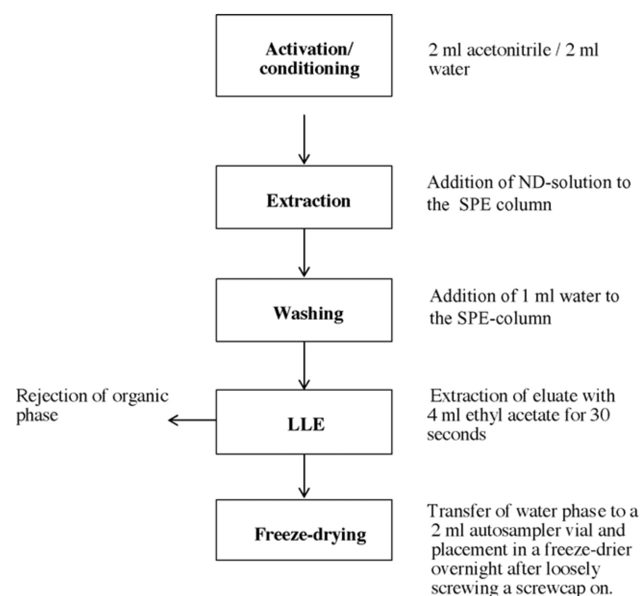


Fig. 2. Final SPE protocol for enrichment of succinic acid in ND solutions.

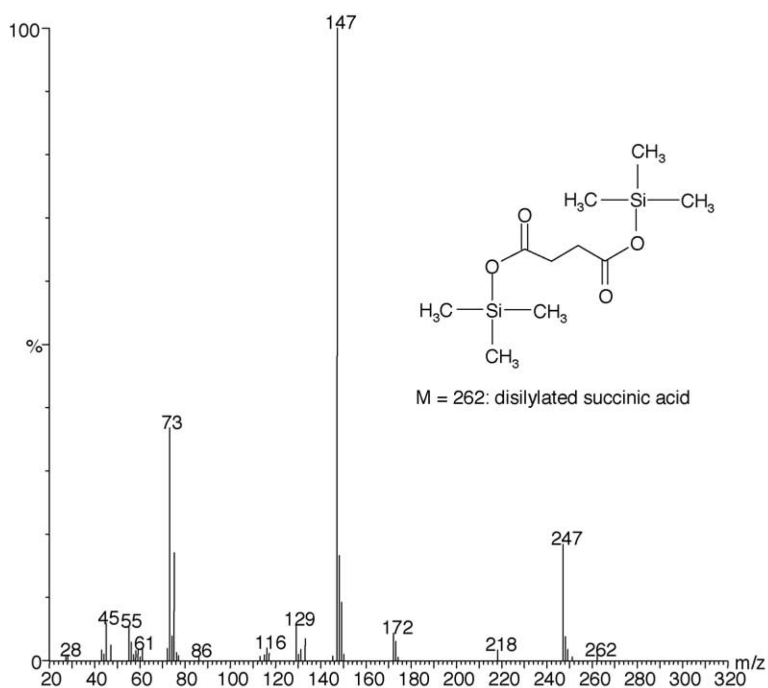


Fig. 3. Mass spectra of disilylated succinic acid. Molecular peak detected at $m/z$ 262. Quantification at $m/z$ 247 in the SIM mode.

acetate for 30 s. The organic phases were then discarded. Two hundred microlitres from each sample was diluted with 1 ml acetonitrile:water (1:1, v/v). Three microlitres of each sample was injected in the HPLC system described above (Section 3.1.1). The results are summarized in Table 3.

Previously, the percentage of NTD trapped and recovered using Isolute 101 columns and freeze drying was determined to be >99%. Based on 4% recovery for LLE in the water phase, almost 100% of NTD would be rejected.

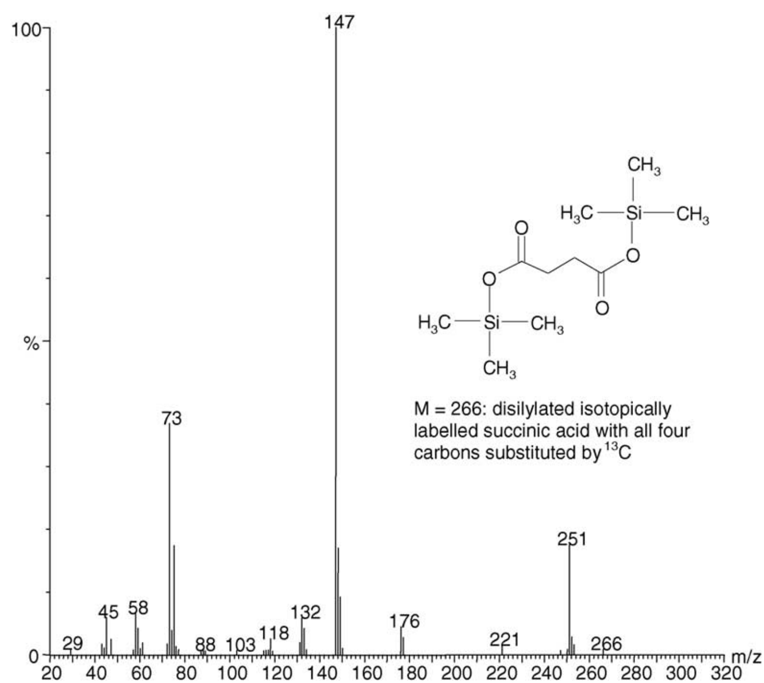


Fig. 4. Mass spectra of disilylated $^{13}$C-labelled succinic acid (IS). Molecular peak detected at $m/z$ 266.Quantification at $m/z$ 251 in the SIM mode.

Table 4
Quantitative results (mean ± SD[a], $n = 2$) of the contents of succinic acid in four ND lots determined by GC–MS analysis

| Lot | Derivative | Concentration in (%, w/w) | RSD (%) |
|---|---|---|---|
| 1 | NTD | 0.210 ± 0.004 | 1.90 |
| 2 | NTD | 0.237 ± 0.004 | 1.68 |
| 3 | NTD | 0.233 ± 0.004 | 1.72 |
| 4 | NAD | 0.176 ± 0.004 | 2.27 |

[a] Pooled SD (degrees of freedom = 6).

### 3.1.4. SPE protocol

See Fig. 2.

## 3.2. Quantitative analysis with GC–MS

The use of a silylating reagent is necessary to make the species volatile, thereby reducing the running time and improving the peak shape for quantification [22]. Silylation of organic acids is a well established-technique [1,2,23,24] and BSTFA is a common silylating reagent that reacts quickly and efficiently, giving consistently reproducible effects [25]. The mass spectra of the trimethylsilyl derivatives of succinic acid and $^{13}$C succinic acid (IS) are shown in Figs. 3 and 4.

The silyl esters of both succinic acid and the isotopically labelled IS have the same retention time, namely 7.9 min. The quantification was performed by monitoring the responses in single ion monitoring (SIM)-mode at $m/z$ 247 (succinic acid) and 251 (IS). Four lots of ND were analyzed, three NTD lots and one NAD lot (Table 4).

### 3.2.1. Linearity of GC–MS method

The linearity of the GC–MS method was tested over succinic acid concentrations ranging from 0.05 to 2.5% (w/w). Injections into the GC–MS system were performed in duplicate. The equation for the regression line of area ratio (succinic acid/IS) on succinic acid in vial (μg) was $y = 0.020x + 0.181$ with a correlation coefficient of 0.9997.

### 3.2.2. Precision

Same-day repeatability studies were performed on the complete SPE, GC–MS method. Six samples from lot 1 were tested (Table 5).

Table 5
Method repeatability within day for NTD, lot 1

| Sample | Sample weight (mg) | Amount detected (μg) | Concentration (%, w/w) |
|---|---|---|---|
| 1 | 10.175 | 21.0 | 0.206 |
| 2 | 9.990 | 21.0 | 0.210 |
| 3 | 9.915 | 20.9 | 0.211 |
| 4 | 9.985 | 21.4 | 0.214 |
| 5 | 10.150 | 21.5 | 0.212 |
| 6 | 10.100 | 21.2 | 0.210 |
| Mean | | | 0.211 |
| SD | | | 0.00264 |
| RSD (%) | | | 1.25 |

Table 6
Analytical recovery of succinic acid in ND-lots after SPE

| Lot | Derivative | Amount added (μg) | Mean (%) ± SD | RSD (%) |
|---|---|---|---|---|
| 1 ($n = 3$) | NTD | 21.2 | 102.6 ± 1.3 | 1.2 |
| 2 ($n = 4$) | NTD | 21.2 | 101.2 ± 1.4 | 1.3 |
| 3 ($n = 4$) | NTD | 132.4 | 99.8 ± 0.7 | 0.7 |
| 4 ($n = 4$) | NAD | 132.4 | 101.8 ± 0.6 | 0.5 |

### 3.2.3. Recovery

The method of adding known standards to working solutions was used to calculate recoveries. The ND batches that had previously been analysed with respect to succinic acid content were chosen as suitable solutions (Table 4). Analyte standards at two concentration levels were spiked into the working solutions prior to SPE. The spiking levels were one and five times by weight (Table 6). From the difference in the amounts of succinic acid standard in the solutions determined before and after spiking, and the known amount added to the matrix, the recovery (%) was calculated.

# 4. Conclusions

The SPE protocol developed in this study provides a suitable sample preparation method for GC–MS analysis of residual succinic acid in nucleoside derivatives. Isolute 101 is a suitable sorbent for this application. The sorbent traps the derivatives efficiently and permits GC–MS analysis of samples enriched with succinic acid. The SPE/GC–MS method yields quantitative results with good repeatability and precision, and is well suited for quality control purposes.

# Acknowledgements

The authors thank Thomas Popoff at Sorbent AB, Sweden, for valuable help in choosing SPE sorbents and Lars Holmberg at Amersham Biosciences for reviewing of the manuscript.

# References

[1] I. Molnár Pearl, A. Vasanits, K. Horváth, Chromatographia 48 (1998) 111.
[2] Y.J. Park, K.R. Kim, J.H. Kim, J. Agric. Food Chem. 47 (1999) 2322.
[3] L.K. Ng, P. Lafontaine, J. Harnois, J. Chromatogr. A 873 (2000) 29.
[4] M. Cocchi, P. Lambertini, D. Manzini, A. Marchetti, A. Ulrici, J. Agric. Food Chem. 50 (2002) 5255.
[5] R.M. Patel, D. Hometchko, G. Marshall, Am. Lab. 2 (1990) 92.
[6] K. Glowniak, G. Zgórka, M. Kozyra, J. Chromatogr. A 730 (1996) 25.
[7] L.A. Berrueta, B. Gallo, F. Vincente, Chromatographia 40 (1995) 474.
[8] R. Jain, K. Lal, H. Bhatnagar, Eur. Polym. J. 22 (1986) 993.
[9] R. Jain, K. Lal, H. Bhatnagar, Thermochim. Acta 117 (1987) 187.

[10] M. Sairam, B. Sreedhar, D.V. Mohan Rao, S. Palaniappan, Polym. Adv. Techn. 14 (2003) 477.
[11] M. MacCoss, D. Cameron, Carbohydr. Res. 60 (1978) 206.
[12] A. Lindström, A.C. Albertsson, M. Hakkarainen, J. Chromatogr. A 1022 (2004) 171.
[13] A. de Villiers, F. Lynen, A. Crouch, P. Sandra, Chromatographia 59 (2004) 403.
[14] S. Suárez-Luque, I. Mato, J. Hhuidobro, J. Simal-Lozano, J. Chromatogr. B 770 (2002) 77.
[15] S. Weigel, R. Kallenborn, H. Hühnerfuss, J. Chromatogr. A 1023 (2004) 183.
[16] Catalogue of Sample Preparation Products and Services, International Sorbent Technologies, 2001, p. 18.
[17] R.C. Denney, Spec. Chem. Mag. 3 (6–7) (1983) 12.
[18] D.R. Lide (Ed.), Handbook of Chemistry and Physics, CRC Press, New York, 1997, p. 363.
[19] T. Stimpfl, J. Jurenitsch, Vycudilik, J. Anal. Toxic. 25 (2001) 125.
[20] R.C. Baselt, R.H. Cravey, Disposition of Toxic Drugs and Chemicals in Man, Chemical Toxicology Institute, Foster City, CA, 1995.
[21] P.J. Andrews, M.A. Quilliam, B.E. McCarry, D.W. Bryant, D.R. McCalla, Carcinogenesis 7 (1986) 105.
[22] K. Blau, G. King, Handbook of Derivatives for Chromatography, Heyden & Sons, London, 1979.
[23] P. Duez, A. Kumps, Y. Mardens, Clin. Chem. 42 (1996) 1609.
[24] S. Tisza, P. Sass, I. Molnár-Perl, J. Chromatogr. A 676 (1994) 461.
[25] Application Note 111 Supelco Bellefonte, PA, 1996.