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Determination of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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Abstract

A sensitive and specific assay based on combined liquid chromatography mass spectrometry (LCMS) has been developed and validated for the quantification of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma. Sample preparation consisted of an extraction with ethyl acetate under basic conditions. The organic solvent was evaporated and the residue re-dissolved for analysis. The extracts were chromatographed on a base deactivated C-8 column interfaced via the heated nebulizer probe to a corona discharge chemical ionization source. The mass spectrometer was operated in the positive ion tandem mode. Typical retention times were 1.5 and 2.0 min for 2-CdA and a fluorinated analog internal standard (IS), respectively. The standard curve was linear from 0.1 to 20 ng ml^{-1} using a 1.0 ml sample volume. The resulting chromatograms produced sharp peaks for 2-CdA and the IS and showed no endogenous peaks from blank plasma. Peak area ratios of 2-CdA to IS were used for standard curve regression analysis. This assay procedure gave interday mean accuracy results for the standards and quality controls that were within 4.9% of target concentrations and interday precision results (RSDs) that were less than 5.3%. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: 2-Chlorodeoxyadenosine, cladribine, 2-CdA; Plasma; Analysis; Liquid chromatography–mass spectrometry

1. Introduction

2-Chlorodeoxyadenosine (cladribine; 2-CdA, Fig. 1) is a purine analog resistant to the action of adenosine deaminase. It is cytotoxic to both resting and proliferating lymphocytes [1,2], which may be especially important in the treatment of indolent lymphoproliferative disorders such as hairy cell leukemia [3,4], chronic lymphocytic leukemia [5–7], and low-grade non-Hodgkin's lymphoma [8–10]. LEUSTATIN™ (2-CdA) has been approved in the United States for the treatment of hairy cell leukemia as a single 7-day continuous intravenous infusion at a dose of

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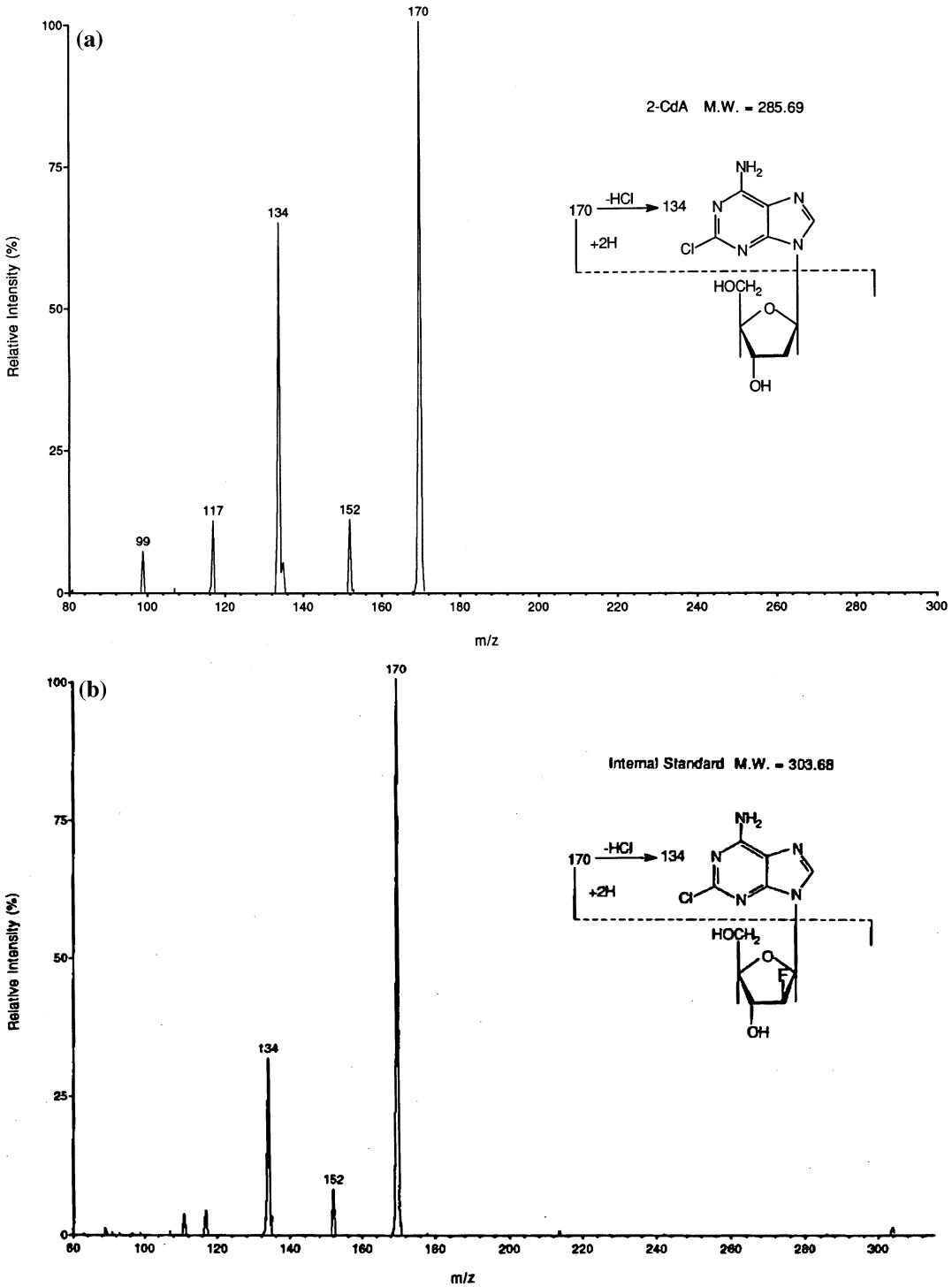

Fig. 1. Structures and product ion mass spectra of (a) 2-CdA and (b) the internal standard. Spectra were acquired in the positive ion mode at the protonated molecular ion for each compound.

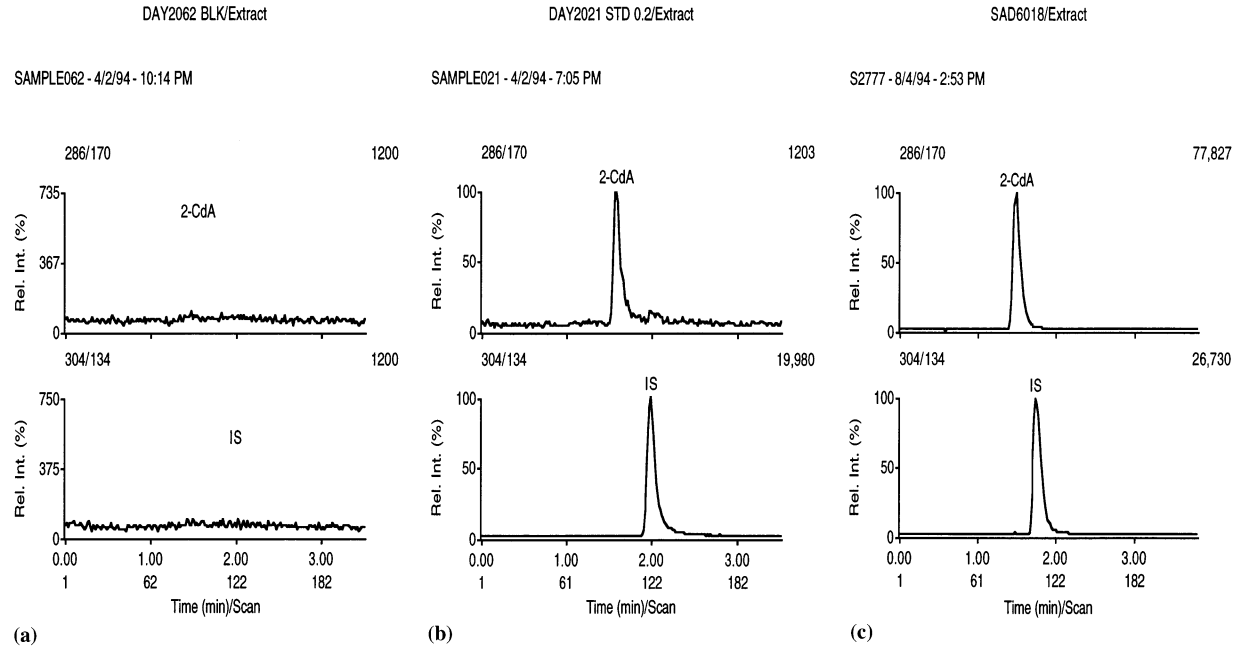

Fig. 2. Representative chromatograms from extracted human plasma samples (A) blank plasma, (B) 0.2 ng ml^{-1} plasma standard and (C) study sample collected 1.0 h after dosing with 0.28 mg kg^{-1} 2-CdA. The transition of 286–170 m/z was monitored for 2-CdA and the transition of 304–134 m/z was monitored for the internal standard.

0.09–0.10 mg kg^{-1} day^{-1} for 5 days. A very sensitive and specific method for the determination of 2-CdA in human plasma with acceptable precision and accuracy at concentrations of 0.1–20 ng ml^{-1} was needed to support clinical studies. Previously published methods for the quantitation of 2-CdA in plasma were based on radioimmunoassay (RIA) [11,12] or high performance liquid chromatography (HPLC) [13,14]. These methods had stated limits of detection of 1 nM (0.29 ng ml^{-1}), but precision and accuracy calculations were only quoted at concentrations down to 10 nM (2.9 ng ml^{-1}). The combination of HPLC with atmospheric pressure chemical ionization (APCI) and tandem MS–MS detection has been extensively used for the low level quantitation of pharmaceuticals in biological matrixes [15–17]. This sensitive and specific technique allowed for both sub ng ml^{-1} quantitation and rapid sample throughput.

2. Experimental

2.1. Materials

2-CdA (RWJ-26251) was obtained from Raylo Chemical (Laporte Fine, Canada). The Internal Standard, RWJ-29727 (6-amino-2-chloro-9-(2-deoxy - 2 - fluoro - beta - *D* - arabinofuranosyl) - 9H - purine, Fig. 1) was synthesized at R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ). Ammonium acetate (HPLC grade) and sodium carbonate (ACS grade) were obtained from Fisher Scientific (Malvern, PA). Methanol (Burdick and Jackson High Purity) was obtained from Baxter (Edison, NJ) and ethly acetate (nanograde) was obtained from EMCSO (Philadelphia, PA).

2.2. Preparation of standards and quality controls

Stock solutions of 2-CdA and the internal standard were prepared by dissolving the compounds

Table 1
Intraday precision for human plasma standards

Nominal concentration of 2-CdA (ng ml^{-1})	Mean peak area ratio	Number of replicates (n)	RSD (%)[a]
0.1	0.0242	6	7.5
0.2	0.0499	5	4.0
0.3	0.0763	6	12.0
0.5	0.122	6	4.0
1	0.241	6	3.7
2	0.476	6	9.1
5	1.24	6	4.7
10	2.52	6	4.9
15	3.64	6	4.7
20	4.89	6	5.4

[a] Relative standard deviation.

in methanol to a concentration of 0.2 mg ml^{-1}. Working standard solutions were prepared by diluting the stock solutions with methanol-water (20:80, v/v). Plasma standards were prepared by adding 50 μl of the appropriate working standard solution to 1 ml of human plasma to yield a standard curve range of 0.1–20 ng ml^{-1}. Quality controls were prepared at 0.5, 2.0 and 10 ng ml^{-1} by adding 0.5 ml of an appropriate working solution to a 49.5 ml human plasma pool. Each quality control pool was stored in 1.4 ml aliquots at −20°C and thawed just prior to analysis.

2.3. Extraction procedure

Fifty μl of the appropriate standard solution and 50 μl of the internal standard solution (1.0 μg ml^{-1}) were added to 1.0 ml of human plasma. After a 2 s vortex, 0.5 ml sodium carbonate (1 M) and 7 ml ethyl acetate were added to each sample. Samples were then mixed using a multitube vortexer for 90 s and centrifuged for 5 min at 2000 rpm. The aqueous layer was frozen by placing the samples on a bed of dry ice and the organic layer was then decanted into a separate tube and evaporated to dryness under nitrogen. The samples were reconstituted with 100 μl of methanol–water (25:75, v/v).

2.4. Analysis procedure

HPLC was performed on a Supelcosil LC-8-DB

Table 2
Interday precision and accuracy for human plasma standards

Nominal concentration of 2-CdA (ng ml^{-1})	Mean calculated concentration (ng ml^{-1})	Number of replicates (n)	RSD (%)[a]	Accuracy (%)[b]
0.1	0.100	6	2.1	100.2
0.2	0.199	6	2.4	99.6
0.3	0.301	6	1.9	100.5
0.5	0.499	6	1.5	99.8
1	1.00	6	1.9	100.2
2	2.04	6	1.8	102.1
5	5.06	6	2.3	101.3
10	10.0	6	3.0	100.4
15	15.1	6	1.9	100.6
20	19.3	6	2.0	96.5

[a] Relative standard deviation.
[b] Accuracy = mean calculated concentration ÷ nominal concentration × 100.

Table 3
Interday precision and accuracy for human plasma quality controls

Nominal concentration of 2-CdA (ng ml^{-1})	Mean calculated concentration (ng ml^{-1})	Number of replicates (n)	RSD (%)[a]	Accuracy (%)[b]
0.5	0.524	12	5.2	104.8
2	2.09	12	3.0	104.4
10	10.4	12	1.9	104.0

[a] Relative standard deviation.
[b] Accuracy = mean calculated concentration ÷ nominal concentration × 100.

3.3 cm × 4.6 mm, 3 μm analytical column with a Supelcosil LC-8-DB 2 cm guard column using a Hitachi L-6200 pump and a Hitachi AS-4000 autosampler. The mobile phase was methanol-5 mM ammonium acetate (25:75, v/v) and the flow rate was 1.0 ml min^{-1}. The volume of the reconstituted extract injected into the HPLC was 30 μl. Tandem MS–MS was performed on a PE-Sciex API III^{Plus} triple-quadrupole mass spectrometer operated in the positive ion mode. The HPLC was interfaced to the mass spectrometer via the heated nebulizer probe with a corona discharge chemical ionization source. The temperature of the nebulizer probe was 450°C. Boil-off from a liquid nitrogen Dewar was used as the nebulizing gas and was maintained at a pressure of 80 psi in the probe. The curtain gas was ultra-high-purity (UHP) nitrogen supplied at a flow rate of 1.5 l min^{-1}. The corona discharge needle was set to + 3 μA and the orifice potential to + 50 V. The mass spectrometer was operated in the multiple reaction monitoring mode (MRM) using argon as the collision gas at a thickness of 325×10^{12} molecules cm^{-2} and a collision energy of 22 eV. The transition of the protonated molecular ion (m/z 286 for 2-CdA and m/z 304 for the internal standard) to a fragment product ion (m/z 170 for 2-CdA and m/z 134 for the internal standard) was monitored using a dwell time of 300 ms. The full product ion spectra for 2-CdA and the internal standard are shown in Fig. 1. Peak area measurements were determined using the PE-Sciex software package MacQuan. The peak area ratios of the standards were used to obtain regression equations and concentrations in quality controls and study samples were then calculated using measured peak area ratios and the regression equation.

3. Results

A precise, accurate and specific method for the determination of 2-CdA in human plasma was validated from 0.1 to 20 ng ml^{-1}. The chromatograms showed no endogenous interference peaks in blank plasma (Fig. 2). The extraction efficiency at 1.0 and 10 ng ml^{-1} was determined by comparing the peak areas of extracted standards against reference samples. The mean extraction efficiencies were 70.8% (RSD = 5.9%, $n = 6$) for 2-CdA and 82.1% (RSD = 3.7%, $n = 6$) for the internal standard. Frozen quality control samples were found to be stable for at least three freeze–thaw cycles.

3.1. Precision and accuracy

The assay was validated for intraday precision by running six replicates of each standard concentration (0.1–20 ng ml^{-1}) on a single day. The relative standard deviations (RSDs) of the peak area ratios were less than 12.1% across the standard curve range and are shown in Table 1. The assay was validated for interday precision, accuracy and linearity by running six standard curves

Table 4
Regression parameters for six standard curves

Curve number	Slope	Intercept	Corr. coef. (r)
1	0.227	0.00458	0.9997
2	0.222	0.00449	0.9994
3	0.222	0.00081	0.9997
4	0.229	0.00049	0.9997
5	0.235	0.00302	0.9996
6	0.238	0.00145	0.9993

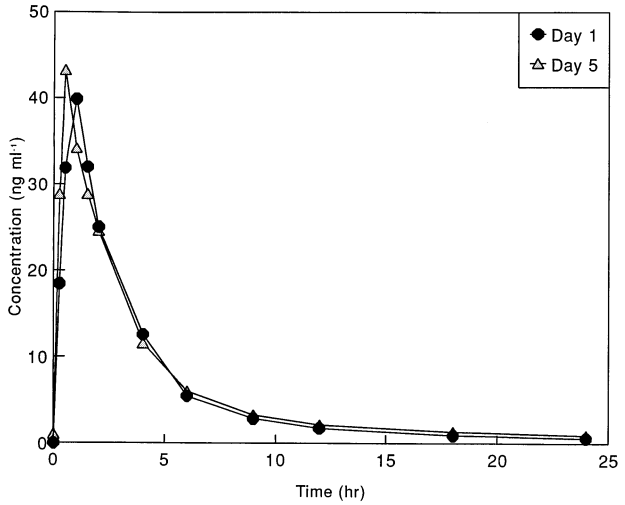

Fig. 3. Mean human plasma concentration–time profiles ($n = 10$) of 2-CdA on days one and five following the oral administration of 0.28 mg kg^{-1} day^{-1}.

over 3 days along with six quality controls with each standard curve. All RSDs were less than 5.3% and all mean accuracy (mean calculated concentration ÷ nominal concentration × 100%) values were within 96.5–104.8% for both the standards and quality controls. The assay limit of quantitation was 0.1 ng ml^{-1}. The results for the standards and quality controls are shown in Tables 2 and 3 respectively. The regression parameters for each standard curve are shown in Table 4.

4. Discussion

This assay offered a 50 fold improvement in sensitivity over a previously applied HPLC–UV method used in our laboratory. Additionally, the high selectivity of tandem MS–MS detection eliminated endogenous interference peaks that were present in the HPLC–UV method, minimized sample preparation and allowed for retention times of less than 2.1 min. Approximately 70 samples were assayed per day with a 4.0 min injection to injection run time. Excellent precision and accuracy were obtained down to concentrations of 0.1 ng ml^{-1}. Linear response started to decline at concentrations over 20 ng ml^{-1}, and analysis of study samples with anticipated concentrations over 20 ng ml^{-1} required dilution. The assay has been demonstrated to be extremely rugged and has been used successfully for the analysis of both clinical and preclinical samples (human, mouse, rat, rabbit, dog and monkey). The assay was found to be free from any interference from known metabolites. Fig. 3 shows the mean ($n = 10$) plasma concentrations of 2-CdA obtained from LCMS analysis following an oral dose of 0.28 mg kg^{-1} day^{-1} for 5 days to human patients [18]. LCMS technology has been successfully applied to the quantitative analysis of many pharmaceutical compounds within our laboratory and has been found to significantly shorten methods development time and increase assay sensitivities.

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