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Identification and quantitation of extractables from cellulose acetate butyrate (CAB) and estimation of their in vivo exposure levels

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Abstract

The purpose of this study was to qualitatively and quantitatively determine potential cellulose acetate butyrate (CAB) extractables in a way to meaningfully predict the in vivo exposure resulting from clinical administration. Extractions of CAB-381-20 were performed in several solvent systems, consistently resulting in the detection of three extractables. The extractables have been identified as acetic acid, butyric acid, and *E*-2-ethyl-2-hexenoic acid (*E*-EHA) by LC/UV, LC/MS and NMR. Extraction studies of CAB powders in acetonitrile/phosphate buffer demonstrated quantitative extraction in 1 h for acetic acid (∼150 μg/g), butyric acid (∼200 μg/g), and EHA (∼20 μg/g). Subsequently, extraction studies for CAB powders and coated tablets in USP simulated gastric and intestinal fluids were performed to evaluate potential in vivo exposure. Similarly, acetic and butyric acids were quantitatively extracted from CAB-381-20 powder after 24 h exposure in both USP simulated fluids. The amounts of EHA extracted from CAB powder after 24 h were determined to be 2 and 16 μg/g in USP simulated gastric and intestinal fluids, respectively. After 24 h exposure in USP simulated fluids, the maximum amount of EHA extracted corresponds to <0.3 μg of EHA per tablet. Pepsin and pancreatin in USP simulated fluids had no effect on EHA extraction and quantitation.

Keywords: Cellulose acetate butyrate; Extractable; 2-Ethyl-2-hexenoic acid

1. Introduction

Most pharmaceutical products are often largely composed of non-drug ingredients, i.e. excipients. The qualification of an excipient for use in a pharmaceutical product includes an assessment of the biological absorption and exposure of the excipient

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Table 1
Acetyl, butyryl and hydroxyl content for CAB powders

CAB ID	% Acetyl (w/w)	% Butyryl (w/w)	% Hydroxyl (w/w)
CAB 381-20	13.5	37	1.8
CAB 171-15PG	29.5	17	1.1

or potential impurities that an excipient may present. The definitive assessment of the biological safety of an excipient is governed by compendial guidances and largely involves a biological test approach. However, a qualitative and quantitative knowledge of the chemical identity of potential impurities can provide added confidence to subsequent quality assessment and estimates of in vivo exposure levels [1–5].

Cellulose acetate butyrate (CAB) has seen increased use as hard contact lenses [6], coating materials [7,8], transdermal patches [9–11], or sustained-release carriers to impact release performance for pharmaceutical solid oral dosage forms [12–18]. Numerous types of CAB are commercially available, differing in their polymer chain length and acetyl, butyryl and hydroxyl content; values for two CAB products that are discussed in this paper are summarized in Table 1. The type of CAB chosen in a pharmaceutical formulation design is largely driven by the functional performance desired in the formulation. Although CAB is widely used in pharmaceutical dosage forms, the literature is devoid of any comprehensive evaluation of extractables. Hydrolytic cleavage of ester linkages present would result in both acetic and butyric acid impurities. Furthermore, the hydrolysis of CAB with a mixture of DMSO/sodium hydroxide/methanol is used to determine acid substituents [19].

The purpose of this study was to assess the potential for other impurities in CAB. Extraction studies were performed as part of the qualification process for several batches of CAB intended for use in experimental drug formulations. As expected, both acetic and butyric acid were detected. Additionally, a relatively hydrophobic, late-eluting peak in the HPLC/UV profile was also observed. This paper describes the detection, isolation, identification and quantification of the compound responsible for this late-eluting peak. Furthermore, qualitative and quantitative determination of all potential extractables from two types of CAB (CAB 381-20 and CAB 171-15PG) is presented in a way to meaningfully predict the in vivo exposure resulting from clinical administration.

2. Experimental

2.1. Materials

Cellulose acetate butyrate (CAB 381-20 and CAB 171-15PG) was purchased from Eastman Chemical Company (Kingsport, TN, USA). CAB (CAB 381-20) coated placebo tablets (average coating weight is approximately 43 mg) were obtained from Merck Research Laboratories (West Point, PA, USA). Pepsin (551 units/mg solid) and *trans*-2-ethyl-2-hexenoic acid (*E*-EHA, 95%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Pancreatin was purchased from Acros Organics Co. (Morris Plains, NJ, USA). Saline (0.9%) was purchased from Baxter Healthcare Co. (Deerfield, IL, USA). Acetonitrile and deionized water were obtained from Fisher Scientific Co. (Pittsburgh, PA, USA). All purchased chemicals were used as received. Sep-Pak® C18 solid phase cartridges were purchased from Waters Co. (Milford, MA, USA).

2.2. Sample preparation

2.2.1. Initial screening studies

Samples of 1 g of CAB 381-20 powder were each suspended in 25 ml of 0.9% saline and 0.9% saline/ethanol (19:1 (v/v)), in triplicate. Each sample was then incubated at either ambient, 50 °C or 70 °C for up to 3 days and analyzed at initial, 1, 2, and 3 days by HPLC. Initial samples were prepared by sonicating CAB 381-20/diluent suspensions for 30 min.

2.2.2. CAB full extraction studies

About 1 g of CAB powder (CAB 381-20 and CAB 171-15PG) was weighed accurately into a 100 ml volumetric flask, followed by the addition of 50 ml of 20 mM phosphate buffer (pH 6.6)/acetonitrile (80:20 (v/v)). The suspensions were stirred for 1 h.

2.2.3. CAB extraction in USP simulated fluids at 37 °C

About 1 g of CAB 381-20 powder was weighed accurately into each 60 ml bottle. Then 50 ml of a

fluid were added to each bottle, either USP simulated gastric (30 ml of 0.1 N HCl, 2 g NaCl, 3.2 g pepsin, QS to 1000 ml, pH ∼ 1.2) or intestinal (6.8 g KH_2PO_4, 77 ml 0.2 N NaOH, 10 g pancreatin, QS to 1000 ml in water, pH ∼ 6.8) fluids. CAB 381-20 powders and coated placebo tablets were extracted into two sets of simulated fluids, those with proteins and without proteins. The suspension in each bottle was then placed into a 37 °C incubator at a 30 rpm shaking speed. Sample aliquots were pulled at 0.5, 4, 8 and 24 h and filtered through a 0.2 μm PTFE filter prior to analysis. Samples with placebo tablets (CAB 381-20 coated) were prepared by putting 24 tablets (containing a total of about 1 g of CAB 381-20) in similar 100 ml bottles and again 50 ml of each respective fluid was added. The tablets were then placed into a 37 °C incubator at the same 30 rpm shaking speed. Samples were taken at 24 h for HPLC/UV assay.

2.3. HPLC and IC assay

2.3.1. Initial screening studies

All sample aliquots were filtered through a 0.2 μm PTFE filter prior to HPLC analysis. HPLC/UV analyses were performed on an Agilent 1100 Series system (King of Prussia, PA, USA) equipped with an autosampler and a variable wavelength UV detector with a 10 mm path length. The analytical column was an Alltech Platinum EPS C18 column (250 mm × 4.6 mm) thermostated at 40 °C. The purpose of the initial screening study was to detect all possible extractables. An EPS C18 column was selected due to its good retention for highly polar compounds, e.g. acetic and butyric acids, which were expected to be present. A gradient method using a mobile phase consisting of aq. 0.1% phosphoric acid and acetonitrile at a flow rate of 1.5 ml/min was developed to ensure retention of these highly polar compounds as well as the potentially more hydrophobic species that may be extracted. The acetonitrile content was kept at 5% for 3 min and then linearly increased from 5 to 81% over the next 27 min. The analytes were detected by UV absorbance at 210 nm. The injection volume was 100 μl and runtime was 40 min. Multichrom version 2.1b (Lab Systems, Beverly, MA) was used for the data acquisition. The method was validated by assessing the specificity for samples extracted from 0.9% saline and 0.9% saline/ethanol (19:1 (v/v)) only.

2.3.2. Preparative isolation of EHA and NMR analysis

One gram of CAB 381-20 powder was suspended in 100 ml of acetonitrile/20 mM phosphate buffer, pH 6.5 (20:80 (v/v)). The sample suspension was stirred for 1 h and aliquots were then filtered prior to analysis. The HPLC/UV and data acquisition system used were the same as described in 2.3.1. The analytical column was an Inertsil ODS3 (250 mm × 4.6 mm; 5 μm particles) thermostated at 30 °C. An ODS3 column was selected due to its better retentivity and robustness for hydrophobic compounds, e.g. EHA, than an EPS C18 column. In addition, the peak shape of EHA, which is critical for isolation by fraction collection, is narrower with an ODS3 column than with the EPS C18 column. The mobile phase consisted of acetonitrile/20 mM phosphate buffer, pH 6.5 (20:80 (v/v)) and acetonitrile at a flow rate of 1.5 ml/min. The acetonitrile content was kept at 20% for 2 min, and then linearly increased to 68% over the next 10 min. The analytes were detected by UV absorbance at 220 nm. The injection volume was 400 μl and runtime was 16 min. Seventeen 1 ml fractions of the unknown were collected at the 8 min elution time. About 1 ml solution was used for LC/MS analysis. The method was validated by assessing the specificity for samples extracted from the diluent only.

The acetonitrile content in the combined HPLC fractions was reduced with a stream of nitrogen, then the sample was loaded onto a C18 solid phase Sep-Pak® cartridge. The cartridge was washed with deuterated water; EHA was eluted with 1 ml deuterated methanol (CD_3OD) and placed in an NMR tube. Fractions of "blank" injections were treated similarly to act as a control sample.

NMR experiments were carried out on a Varian^{UNITY}*INOVA* 600 MHz spectrometer with sample maintained at 25 °C. TMS was added as an internal reference for the proton and carbon spectra. The acetonitrile and methanol resonances observed in the control sample were disregarded in the spectrum of the unknown. Proton connectivities were established by simple decoupling experiments. Carbon chemical shifts were obtained from HMQC and gradient-HMBC data.

2.3.3. EHA assay

The HPLC/UV and data acquisition system used were the same as described in 2.3.1. The analytical column was an Inertsil ODS3 column (250 mm × 4.6 mm) thermostated at 40 °C. The mobile phase consisted of acetonitrile/50 mM phosphate buffer, pH 4.5 (50:50 (v/v)) at a flow rate of 1.5 ml/min. The analytes were detected by UV absorbance at 220 nm. The injection volume was 100 μl and runtime was 15 min. An isocratic method was employed because EHA is the only compound of interest and the resolution was known to be sufficient at that point. The method was validated by assessing the specificity, recovery, linearity, measurement precision, and limit of quantitation (LOQ). The recovery and linearity were assessed in both the USP simulated gastric and intestinal fluid across the concentration range of 0.01–0.5 ppm. The mean recovery for EHA in gastric fluid was 100.9% with a relative standard deviation (R.S.D.) of 1.3%. The mean recovery for EHA in intestinal fluid was 101.5% with an R.S.D. of 1.2%. The correlation coefficients (R^2) were 0.99998 and 0.99997 for gastric and intestinal fluids, respectively. The R.S.D. values of the measurement precision for 0.2 and 0.01 ppm EHA standards were 0.2 and 3.5%, respectively. The LOQ was defined as 0.005 ppm with a S/N ratio of 22:1.

2.3.4. Spiking EHA standard with isolated EHA

The isolation of EHA from CAB extractables was achieved by suspending 1 g of CAB 381-20 in 25 ml of acetonitrile/20 mM phosphate buffer, pH 6.6 (20:80 (v/v)) diluent. The suspension was stirred for 1 h and filtered through a 0.2 μm PTFE filter prior to HPLC analysis on an Inertsil ODS3 column (250 mm × 4.6 mm) thermostated at 40 °C. The HPLC/UV and data acquisition system used were the same as described in Section 2.3.1. The mobile phase consisted of acetonitrile/0.1% phosphoric acid (5:95 (v/v)) (A) and acetonitrile (B) at a flow rate of 1.5 ml/min. The acetonitrile content was kept at 5% for 3 min and then increased to 81% over 27 min; the runtime was 40 min. One hundred microliter injection volumes were used and the analytes were detected by UV absorbance at 210 nm. Ten fractions of EHA were collected at the 16.8 min elution time. The isolated EHA was re-injected into the HPLC system. Compared with the preparative isolation method (see Section 2.3.2), the gradient range was expanded from an acetonitrile level of 20–68% to 5%–81% in order to potentially separate the E and Z isomers of EHA. However, only the E isomer is commercially available, and peak splitting of the two isomers was not observed. The method was validated by assessing specificity for samples extracted in the diluent only.

The EHA standard solution was prepared by diluting 10 μl EHA in 100 ml of acetonitrile/20 mM phosphate buffer, pH 6.6 (20:80 (v/v)). The resulting solution was then further diluted 100-fold in the same solvent mixture, to give a 1 ppm EHA standard solution. The co-spiking solution was prepared by mixing 500 μl each of EHA standard solution (1 ppm) and the isolated EHA. The chromatographic conditions were the same as above.

2.3.5. Acetic and butyric acid assay

Ion chromatography (IC) was employed to quantify acetic and butyric acids, since it has better separation for these two compounds than the HPLC methods. The IC analyses were performed with a Dionex GP50 gradient pump (San Jose, CA, USA) equipped with a Thermal Separation AS3500 autosampler and a Dionex ED40 electrochemical detector. The analytical column was an IonPac AS11-HC column (250 mm × 4 mm) equilibrated at ambient temperature. The mobile phase consisted of 1 mM sodium hydroxide solution (A) and 60 mM sodium hydroxide solution (B) at a flow rate of 1.4 ml/min. The B content was kept at 0% for 12 min, increased from 0 to 100% over 1 min, and kept at 100% for 3 min. The analytes were detected with suppressed conductivity detection (ASRS) in the autosuppression external water mode. Injection volume was 10 μl and runtime was 30 min. Several modifications were made for analysis of tablets, affecting the final three timepoints in the gradient (gradient: NaOH content kept at 1 mM for 12 min and then increased from 1 to 60 mM in 1 min and kept at 60 mM for 6 min). The runtime was changed to 40 min thereafter. The method was validated by assessing specificity, linearity, measurement precision, and LOQ. The correlation coefficients (R^2) across the concentration range of 0.5–6 ppm were 0.9886 and 0.9963 for acetic and butyric acid, respectively. The R.S.D.s were 1 and 5% for acetic and butyric acid, respectively. The LOQ for both compounds was defined as 0.5 ppm.

2.4. LC/MS analysis

Aliquots (100–190 μl) of sample solution obtained from the preparative isolation of EHA (see Section 2.3.2) were injected onto a Perkin Elmer 200 Series HPLC system, equipped with a Perkin Elmer 235 UV detector interfaced to a ThermoFinnigan LCQ mass spectrometer. Chromatography was performed on an Alltech Platinum EPS C-18 column (250 mm × 4.6 mm; 5 μm particles) held at 40 °C. The mobile phase was water/acetonitrile (60:40 (v/v)) at a flow rate of 1.0 ml/min. UV detection was at 220 nm and the runtime was 10 min. The LC method was validated by assessing specificity only. For APCI/LC/MS, the corona discharge was set at 4.5 kV and the APCI probe was operated with the vaporizer at 450 °C and the heated capillary at 200 °C. The sheath gas (N_2) and auxiliary gas (N_2) settings were 70 and 30 arbitrary units, respectively. Data were collected in the negative ion mode.

3. Results and discussion

3.1. Initial screening studies

Cellulose acetate butyrate (CAB) polymers are prepared by acetic and butyric acid anhydride esterification of cellulose to varying degrees (Fig. 1). The two types of CAB examined here differ only in their acetyl and butyryl content (Table 1). Initial screening tests performed on CAB 381-20 revealed that three peaks were extracted into either 0.9% saline (Fig. 2) or 0.9% saline/ethanol (19:1 (v/v)) (not shown). Two extractables were readily identified as acetic acid and butyric acid, which are reasonable impurities. The acids could

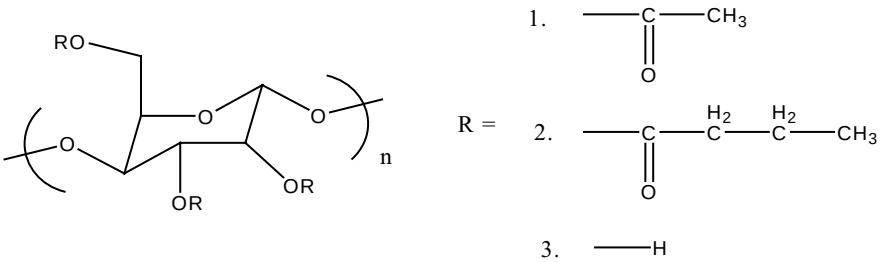

Fig. 1. Molecular structure of cellulose acetate butyrate (CAB) [7].

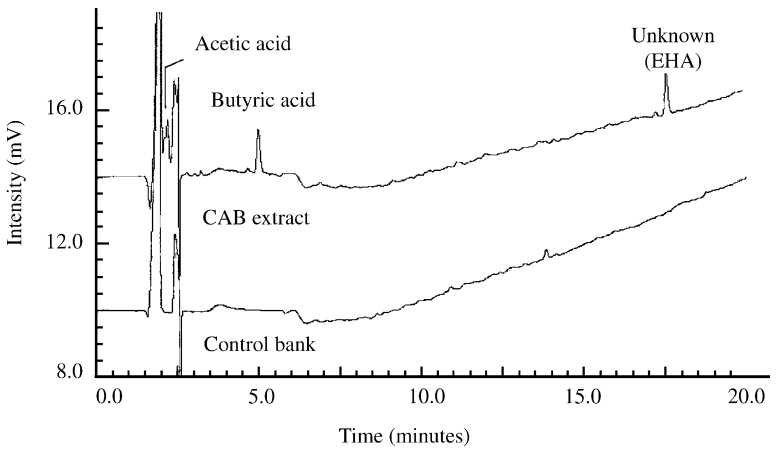

Fig. 2. Chromatograms of CAB extract and control blank in saline (see experimental detail in Section 2.3.1. Only the first 20 min of the chromatograms are shown).

also be potential degradates from the hydrolysis of their respective esters in CAB polymers. The manufacturer confirmed that acetic acid and butyric acid are known impurities in CAB 171-15PG, but was not able to provide any insight into the presence of the unknown impurity in CAB 381-20 and CAB 171-15PG.

Quantitative estimation of the CAB extractables in 0.9% saline and 0.9% saline/ethanol (19:1 (v/v)) was also performed. Accurate acetic acid quantitation was not possible due to significant interference from peaks near the solvent front. Levels of the unknown were initially estimated using a butyric acid standard as a reference. The amounts of butyric acid in CAB 381-20 extracted under ambient and elevated temperature conditions (Table 2) were found to be the same with extraction by 0.9% saline and 0.9% saline/ethanol (19:1 (v/v)). However, the level of the unknown extracted into 0.9% saline/ethanol (19:1 (v/v)) was higher than that in 0.9% saline, which suggests that the unknown is less water-soluble than butyric acid. The apparent higher lipophilicity is also consistent with the observed longer chromatographic retention times.

Table 2
Extraction levels for CAB 381-20 in 0.9% saline and 0.9% saline/ethanol (19:1 (v/v))

Stress condition	Extractant	Day	Butyric acid (μg/g or ppm)	Unknown[a] (μg/g or ppm)
Ambient	Saline	0	111	100
		1	106	81
		3	137	85
50 °C	Saline	0	111	100
		1	110	102
		3	143	165
	5% EtOH in saline	0	124	195
		1	109	175
		3	140	258
70 °C	Saline	0	111	100
		1	143	162
		3	235	366
	5% EtOH in saline	0	124	195
		1	118	261
		3	206	477

[a] Quantitated using butyric acid as a reference standard, with RRF = 1 for butyric acid vs. EHA.

3.2. Molecular characterization of the unknown

The chromatographic behavior of the unknown resulting from pH changes in the mobile phase clearly indicated that an acid functionality was present. Furthermore, the UV absorption spectrum of the unknown is very similar to that of acetic and butyric acids, but exhibits an approximately 10 nm red-shift (Fig. 3). Initial LC/MS experiments were performed utilizing both electrospray (ESI) and atmospheric pressure chemical ionization (APCI) in either positive or negative ion modes. Aqueous formic acid (0.1%) and ammonium

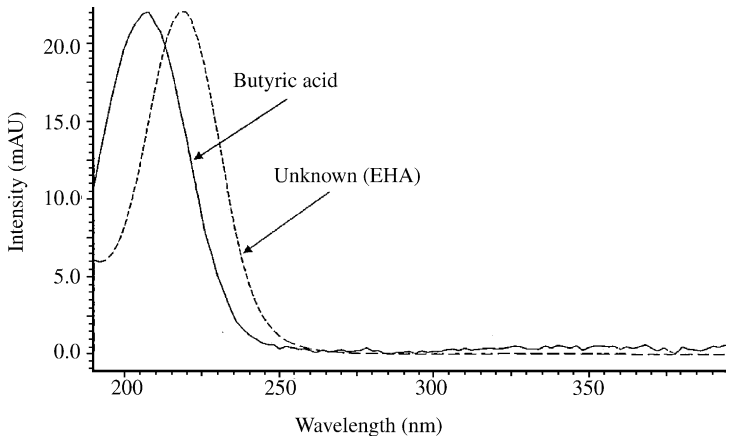

Fig. 3. UV spectra of butyric acid and the unknown (EHA). The UV spectra were measured directly from the CAB extract sample shown on Fig. 1 by the diode array detector of the HP1100 chromatographic system. The chromatographic conditions are the same as in Fig. 1.

acetate (10 mM, pH 5) were used as mobile phases. However, a reproducible molecular weight could not be obtained under these conditions due to the extremely weak signal from the unknown peak. Failure to detect an $[M+H]^+$ signal in the positive ion mode suggested the probable absence of N from the molecule. A molecular weight of 142 Da ($[M-H]^-$ at m/z 141) was determined in the absence of added buffer in the mobile phase, utilizing MS in the negative APCI mode where background signals were dramatically reduced. Assuming that the molecule contained only carbon, oxygen, hydrogen with one –COOH group, the molecular weight suggested one unsaturated carbon-carbon bond, which in conjugation with the acid group would provide the desired red-shift in the UV-Vis spectrum.

The unknown was then isolated and characterized by NMR. The NMR analysis showed the unknown to be a salt of 2-ethyl-2-hexenoic acid (EHA; see Fig. 4), consistent with the presence of an acid group and a molecular weight of 142 Da for the free acid. Propyl and ethyl group fragments were readily identified by inspection of the 1D proton spectrum and decoupling experiments. A 6.31 ppm hydrogen atom was found to be directly attached to an unsaturated carbon with a chemical shift of 137.3 ppm (HMQC data). This olefinic hydrogen was found to be coupled to the 2.11 ppm CH_2 of the propyl group suggesting that the propyl fragment is attached to a CH=C fragment. Direct attachment to the olefinic CH was verified by observation of an HMBC correlation from the 1.44 ppm CH_2 of the propyl group to the 137.3 ppm carbon. A similar analysis showed that the ethyl fragment was attached to the other side of the double bond (141.0 ppm). The presence of a carboxylate carbon (178.5 ppm) was determined by HMBC correlations from the olefinic (6.31 ppm) and ethyl group CH_2 (2.26 ppm) proton resonances. Attempts to observe NOE across the double bond were unsuccessful due to the limited amount of sample. The regiochemistry of the double bond was later determined to be E by NMR comparison with authentic E and Z-isomers of EHA (see later).

Seven commercially available carboxylic acids of molecular weight 142 Da (including EHA) were then obtained and chromatographed. Spikes of EHA into the CAB extractables solution showed co-elution with the unknown (Fig. 5). Furthermore, the commercially obtained *E*-EHA has the same UV spectrum

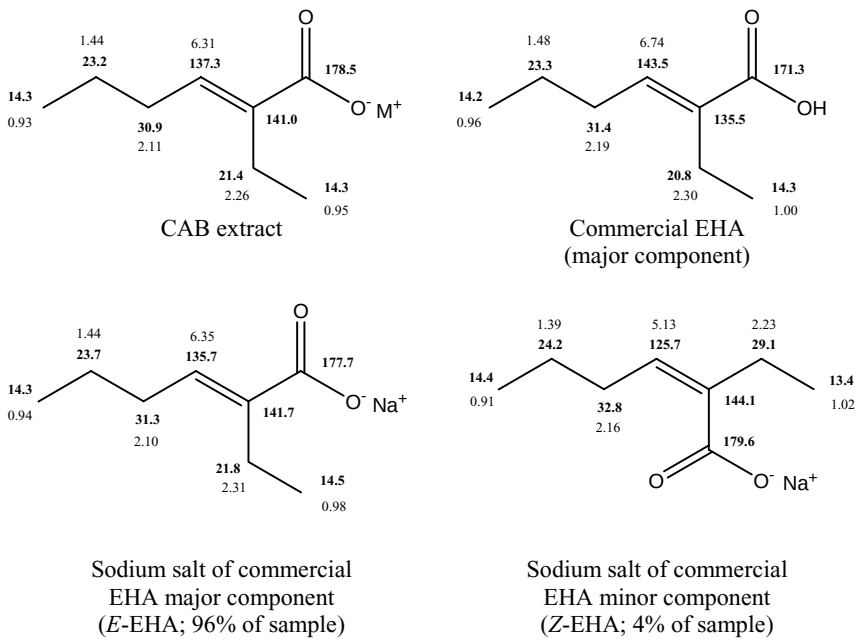

Fig. 4. Structures of the isolated unknown from CAB extract and commercial EHA as determined by NMR. Observed carbon chemical shifts are given in bold font and proton chemical shifts are given in normal font (samples dissolved in CD_3OD; 25 °C).

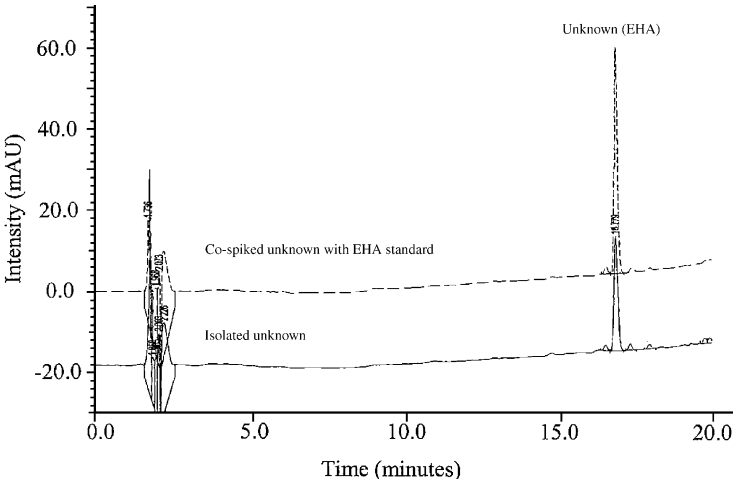

Fig. 5. Chromatograms of the isolated unknown and co-spiked unknown with EHA standard (see experimental detail in Section 2.6. Only the first 20 min of the chromatograms are shown).

(approximately 10 nm red-shifted from butyric acid) and mass spectrum as the unknown CAB extractable. Thus the CAB extractable is confirmed to be EHA.

Proton and carbon NMR chemical shifts of authentic EHA dissolved in CD_3OD match those of the unknown when the EHA is converted to the sodium salt form (Fig. 4). The commercial sample was found to be a 96:4 mixture of E:Z isomers based on integration and NOE experiments. As shown in Fig. 4, chemical shifts of the E-isomer align nicely with those of the CAB extractable. Minor chemical shift differences are attributable to differences in concentration, nature of the counterion(s), base concentration, and/or presence of other solvent molecules in the CAB extract. The CAB extractable is therefore unambiguously determined to be *E*-2-ethyl-2-hexenoic acid. Observation of the salt form is believed to be merely an artifact of the isolation procedure, where aq. phosphate buffer of pH 6.5 was present in the extraction medium.

The source of EHA in CAB is unknown, but it could enter the CAB process as a low level impurity in the butyric acid reagent (unable to confirm from the manufacturer). EHA could also form as a condensation product of butyraldehyde (present as a low-level impurity in the butyric acid [1]) and butyric acid.

3.3. Quantitative extraction data

3.3.1. CAB full extraction

Acetic acid, butyric acid and EHA are believed to be fully extracted from the CAB polymers after 1 h of stirring in 50 ml of 20 mM phosphate buffer (pH 6.6)/acetonitrile (80:20 v/v). The amounts of acetic acid, butyric acid, and EHA are on the order of 100–200, 100–200, and 10–30 μg/g CAB powder in two lots of technical grade CAB 381-20, respectively (Table 3). CAB 381-20 is the grade of CAB that provided the desired functional properties for a specific coated tablet formulation. It is interesting to note that the extractable levels in the pharmaceutical grade of CAB 171-15PG were found to be significantly higher under the same extraction conditions.

Table 3
Estimated total amounts of extractables in different CAB powders

CAB powder	Acetic acid (ppm)	Butyric acid (ppm)	EHA (ppm)
CAB 381-20, Lot# 59792, Technical Grade	110	180	18
CAB 381-20, Lot# 59962, Technical Grade	92	180	21
CAB 171-15PG, Pharmaceutical Grade	280	260	50

Table 4
Amounts of acetic and butyric acid extracted from CAB 381-20 in USP simulated fluids without proteins added

Timepoint (h)	Acetic acid (ppm)		Butyric acid (ppm)	
	Gastric fluid	Intestinal fluid	Gastric fluid	Intestinal fluid
0.5	97	85	110	120
4	99	95	140	140
8	94	90	130	140
24	140	100	190	160

3.3.2. CAB extraction in USP simulated fluids at 37 °C

In light of the total levels of the extractables reported above, it is desirable to estimate biologically relevant levels of each extractable. However, it was found that simulated fluids stressed at 37 °C interfered with the quantitation of acetic acid and butyric acid by HPLC/UV. Therefore, quantitation results for acetic acid and butyric acids were obtained from simulated fluids in the absence of pepsin or pancreatin (Table 4). The majority of the acetic and butyric acid observed after 24 h is present at the 30 min timepoint. The increases observed beyond 30 min are likely a combination of further extraction and some hydrolysis of CAB esters. As expected, the amounts of acetic and butyric acids extracted are comparable to the total amount present (see Table 3).

The amounts of EHA (μg/g or ppm) extracted from CAB 381-20 in both USP simulated gastric fluid and simulated intestinal fluid (with and without proteins) are summarized in Table 5 as a function of extraction time. As expected, the amount of EHA extracted is (1) less than the total amount present (21 μg/g, see Table 3), and (2) more pronounced in the higher pH intestinal fluids, by about a factor of eight. Furthermore, the levels observed are independent of the presence or absence of protein in the extraction media. The data in Table 5 suggest that extraction into these simulated fluids in the absence of proteins represents an accurate reflection of the amounts one would observe in the presence of either pepsin or pancreatin for acetic and butyric acids (Table 4).

3.3.3. Estimated exposure per 24 h oral dosage of CAB coated tablets

The pharmaceutically relevant product at the focus of these investigations is a CAB coated tablet. Therefore, the extractions were also performed for coated tablets in the same simulated gastric and intestinal fluids. Table 6 summarizes the amounts of EHA (μg per tablet) extracted from the CAB 381-20 coated tablets with and without proteins, again as a function of time. The amount of EHA extracted is similar for samples either in the presence or absence of proteins. As noted before, the levels of EHA observed are higher for

Table 5
Amounts of EHA extracted from CAB 381-20 in USP simulated fluids with and without proteins added

Timepoint (h)	EHA (ppm)			
	Gastric fluid with pepsin	Gastric fluid without pepsin	Intestinal fluid with pancreatin	Intestinal fluid without pancreatin
0.5	0.6	1.2	2	3
4	1.6	1.7	7	8
8	1.8	1.9	10	10
24	2.0	1.9	16	15

Table 6
Amounts of EHA extracted from CAB 381-20 coated tablets in USP simulated fluids with and without proteins added[a]

Timepoint (h)	EHA (μg per tablet)			
	Gastric fluid with protein	Gastric fluid without protein	Intestinal fluid with protein	Intestinal fluid without protein
0.5	0.02	0.02	0.04	0.03
4	0.04	0.04	0.10	0.09
8	0.06	0.05	0.15	0.13
24	0.10	0.09	0.27	0.26

[a] Average tablet weight is 614 mg with about 7% CAB coating.

Table 7
Amounts of acetic and butyric acid extracted from CAB 381-20 coated tablets in USP simulated fluids without proteins added[a]

Timepoint (h)	Acetic acid (μg per tablet)		Butyric acid (μg per tablet)	
	Gastric fluid	Intestinal fluid	Gastric fluid	Intestinal fluid
0.5	<LOQ[b]	<LOQ	<LOQ[c]	<LOQ
4	<LOQ	<LOQ	<LOQ	<LOQ
8	<LOQ	<LOQ	1	<LOQ
24	1	1	3	1

[a] Average tablet weight is 614 mg with about 7% CAB coating.
[b] LOQ for both compounds were determined to be 0.5 μg per tablet.
[c] LOQ for both compounds were determined to be 0.5 μg per tablet.

intestinal pH values, however, the levels are approximately a factor of 2–3 lower (on a μg/g CAB basis) than observed for the powders (Table 5).

The amounts of acetic acid and butyric acid extracted from these tablets in the absence of proteins are shown in Table 7. The amounts of acetic and butyric acids observed are approximately a factor of 2–5 lower (on a μg/g CAB basis) than observed for the powders above (Table 4). The lower extractable results are as expected, due to the reduced CAB surface area for the coated tablets compared to the initial powders.

4. Conclusions

Acetic acid, butyric acid, and *E*-2-ethyl-2-hexenoic acid (*E*-EHA) are extractables present in these two grades of CAB (381-20 and 171-15PG). The pharmaceutical grade of CAB (171-15PG) contains higher levels of acetic and butyric acids and EHA than CAB 381-20. Total in vivo exposure over 24 h during administration of a CAB-coated tablet for EHA, acetic acid and butyric acid was estimated to be 0.3, 1 and 3 μg, respectively. The extraction levels are a factor of 2–5 lower in coated tablets than observed in powders (in terms of μg/g CAB).

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