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Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 896–900
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Profiling of medium chain glycerides used in pharmaceutical formulation development by reversed-phase HPLC

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Received 8 July 2005; received in revised form 22 August 2005; accepted 23 August 2005
Available online 27 October 2005

Abstract

Medium chain length (C8, C10) mono-, di- and triacylglycerols (MCGs) are used in pharmaceutical formulation development of poorly soluble compounds, as a means to increase their oral bioavailability. The ratios of C8 and C10 fatty acid components along with the ratio of monoglycerides, diglycerides and triglycerides can significantly impact overall solubilizing characteristics both in vitro and in vivo. Existing literature methods either do not have the desired selectivity or simplicity to adequately characterize these MCGs. A reversed-phase HPLC method has been developed utilizing a Waters Symmetry C18 (150 mm × 4.6 mm, 3.5 μm particle size) column with a gradient of acetonitrile and water. The effluent was monitored using a UV detector at 220 nm and relative response factors were determined for all components with available standards to allow for accurate quantitation. The RP-HPLC method was optimized for selectivity, sensitivity and efficiency and was successfully applied to the characterization of commercial samples yielding superior sensitivity and ease of preparation compared to existing compendial methods.

Keywords: Medium chain glycerides; FAMES assay; HPLC-UV

1. Introduction

A common strategy to maximize the oral bioavailability of poorly soluble drugs used in the pharmaceutical industry is to solubilize the drug in pharmaceutically acceptable surfactants [1]. One common surfactant seeing broader use is medium chain glycerides, which are mixtures of monoglycerides, diglycerides, and triglycerides of caprylic (C8) and caproic (C10) acids. Medium chain glycerides are suggested to increase bioavailability in three possible ways. The first mechanism is via their ability to act as an absorption promoter, similar to compounds such as fatty acids, surfactants, steroidal detergents and other muco-adhesive polymers [2]. The second mechanism is through permeability enhanced gastric emptying inhibitory effects [3]. The third mechanism is by improving the API solubility both in the formulation and in vivo [4].

Medium chain glycerides are controlled for use in US and European markets based on their acceptability as determined by United States Pharmacopeia (USP) or European Pharmacopeia (PhEur) characterization tests. Compendial requirements for MCGs are ≥40.0% monoglyceride and ≤7% free glycerin, allowing the rest of the glycerides to be comprised of some ratio of diglycerides, triglycerides and free fatty acids [3]. These composition requirements allow for a wide range of materials with a great deal of flexibility to fall within the acceptance criteria for a MCG. The USP and PhEur methods for monoglycerides and diglycerides utilize a gel permeation chromatography (GPC) method that provides minimal separation of glycerol, monoglyceride, diglyceride, and triglyceride components and offers little information regarding the chain length of these components [3,4]. These pharmacopeial methods are inadequate in characterizing the fine details of fatty acid constituents. Since the degree of absorption modification by MCGs is correlated with the ratios of mono-, di-, and triglycerides as well as the ratios of caprylic versus caproic chain lengths [5], it is desirable to have a method with improved selectivity for determining these MCG qualities.

Extensive research exists [6–9] using HPLC coupled with various detectors (RI, ELSD, APCI-MS) to separate and quantify mixtures of triglycerides. Most of these separations can be accomplished by isocratic elution due to the similar elutotropic properties of the triglycerides, but do not address samples that contain monoglycerides and diglycerides. Adequate analysis of

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doi:10.1016/j.jpba.2005.08.024

MCGs has been achieved by gas chromatography with detection by flame-ionization detection, but this technique requires an undesirable methyl esterification or silylation step along with quantitation by an internal standard [10–12].

The procedure presented here is a simple reverse phase HPLC system with UV detection to analyze samples containing various mixtures of MCGs. This method adequately separates all of the desired molecular fragments contained in three commercially available samples of MCG while utilizing a universal and readily available detector for analysis. The procedure utilizes external standards to accurately quantitate individual components, total monoglycerides, total diglycerides, total triglycerides, total free fatty acids and caprylic–caproic ratios. The relative response factors of the mixtures have been calculated allowing for their quantitation without the purchase of individual standards. This method serves as a vital analytical tool for the full characterization and monitoring of MCGs to be used in pharmaceutical formulations.

2. Experimental

2.1. Materials

Solvents used were of HPLC grade or equivalent and were purchased from the following sources acetonitrile, *o*-phosphoric acid (85%), and glacial acetic acid from Fisher Scientific (Fair Lawn, NJ, USA); isopropanol from Mallinckrodt. Components of MCG mixtures were all of ∼99% purity and purchased from the following sources and used as received: 1-monooctanoylglycerol, 1-monodecanoylglycerol, decanoic acid (free acid), octanoic acid (free acid), 1,2-dioctanoylglycerol, 1,2-didecanoylglycerol, tridecanoylglycerol, and trioctanoylglycerol (Sigma Chemical Co., St. Louis, MO). Imwitor 742 and Miglyol 812 (Sasol Corp., Houston, TX) and Capmul MCM (Abitec Corp., Janesville, WI.)

2.2. HPLC-UV instrumentation and conditions

The HPLC-UV study was performed with an Agilent (Wilmington DE) Model 1100 equipped with a UV–vis VWD (1 cm cell). A Waters Symmetry® C18 150 mm × 4.6 mm (3.5 μm) column with a flow rate of 1.0 mL/min was used for the separation of 50 μL injections. The column temperature was maintained at 40 °C and detection was at 220 nm. A gradient of acetonitrile and *o*-phosphoric acid (0.1%) was employed to obtain suitable separation. The linear gradient used was as follows: H_3PO_4 (0.1%):ACN (70:30, v/v); from 0 to 10 min to H_3PO_4 (0.1%):ACN (25:75, v/v), from 10 to 20 min to H_3PO_4 (0.1%):ACN (20:80, v/v), from 20 to 22 min to H_3PO_4 (0.1%):ACN (1:99, v/v), then held for 23 min. A re-equilibration time of 5 min was employed between injections.

2.3. HPLC-MS instrument conditions

LC/MS identification was performed using a Hewlett-Packard Model 1100 HPLC system connected to a Thermo-Finnigan LCQ MS using the same conditions reported for the HPLC-UV experiments with the exception the *o*-phosphoric acid being replaced by acetic acid (0.05%). The MS utilized APCI with an inlet temperature of 250 °C and a capillary temperature of 150 °C. The corona voltage was set to 5.0 μA throughout. Spectra were obtained from *m*/*z* 50 to 700 with a scan time of 1.75–2.00 s. Chromatograms were processed using 7-point smoothing and all mass spectra were obtained using an average over the breadth of the chromatographic peak.

2.4. Standard and sample preparation

1-Monodecanoylglycerol was used as the external standard for quantification. This component of the MCGs was chosen as the standard because of a clean baseline around the peak allowing for consistent integration and the inexpensive cost to obtain a high purity sample. A working standard was prepared at a concentration of 1 mg/mL using IPA:H_3PO_4 (0.1%) (70:30, v/v) as the diluent. Relative response factors were empirically determined (Section 3.2) for each of the other commercially available components of MCGs, correcting for purity, to allow for accurate quantification. For commercially available samples of MCG, a 5 mg/mL solution was prepared in IPA:H_3PO_4 (0.1%) (70:30, v/v). The samples and standards were injected without further clarification. Total glyceride calculations were performed by summing the individual components.

3. Results and discussion

3.1. Identification of MCG components

The primary components of MCGs are acylglycerols with combinations of eight and ten carbon side chains. Depending on the manufacturing process, it is possible that the samples will contain low levels of acylglycerols with chain lengths other than eight or ten carbons. The acylglycerols can also hydrolyze to form the corresponding chain length free fatty acid, diglycerides, monoglycerides and glycerol. These molecular fractions can be present in detectable levels depending upon the age and storage condition of the sample. The development of the HPLC separation was initiated with the goal of separating and quantitating the 15 possible compounds present in the samples (Table 1).

Typically, the choice of stationary phases depends on the constituent of MCGs being monitored. Commonly cited phases include phenyl [13], C8 [14] and C18 [15–17]. However, many of these methods focused on separation of triacylglycerols exclusively. Columns with various stationary phases (including C8, C18 and phenyl) from multiple suppliers were examined. The Waters Symmetry® C18 column was chosen with a reduced (3.5 μm) pore size for further method development as it provided a suitable compromise between acceptable resolution and reasonable analysis time. The gradient parameters were chosen with a focus on separating the 1,2- and 1,3-diglyceride components of each chain length. A gradient was necessary to achieve a reasonable run time because of the wide range in hydrophobicities contained in the MCG samples with components ranging from C8 monoglyceride to C10 triglyceride. An analysis wavelength of 220 nm was chosen to reduce noise in the chromatogram that

Table 1
Molecular components of commercially available MCG samples

Chromatographic assignment (Fig. 1)	Molecular assignment	Source purity[a] (%)	Chromophore			Observed MS peaks, *m/z*	Relative response factor, wt.% basis
			Ester group	Alcohol group	Acid group		
Not present	Glycerol	99	0	3	0	61[b] and 43	NA
1	2-Monooctanoyl glycerol	Not available	1	2	0	219[b], 201, 101	1.11
2	1-Monooctanoyl glycerol	~99	1	2	0	219[b], 201, 101	1.11
3	Octanoic acid	>99	0	0	1	145[b], 60, 73, 43	1.20
4	2-Monodecanoyl glycerol	Not available	1	2	0	247[b], 229	1.00
5	1-Monodecanoyl glycerol	~99	1	2	0	247[b], 229	1.00
6	Decanoic acid	>99	0	0	1	173[b], 60, 73, 41, 43	0.99
7	1,2-Dioctanoyl glycerol	~97	2	1	0	345[b], 327, 201	1.48
8	1,3-Dioctanoyl glycerol	Not available	2	1	0	345[a], 327, 201	1.48
9	1-Octanoyl-2-decanoyl glycerol	Not available	2	1	0	373[b], 355, 229, 201	1.39
10	1-Octanoyl-3-decanoyl glycerol	Not available	2	1	0	373[b], 355, 229, 201	1.39
11	1,2-Didecanoyl glycerol	~97	2	1	0	401[b], 383, 229	1.29
12	1,3-Didecanoyl glycerol	Not available	2	1	0	401[b], 383, 229	1.29
13	Trioctanoyl glycerol	>99	3	0	0	471[b], 327	1.43
14	Octanoyl-octanoyl-decanoyl glycerol	Not available	3	0	0	499[b], 355, 327	1.48
15	Octanoyl-decanoyl-decanoyl glycerol	Not available	3	0	0	527[b], 383, 355	1.54
16	Tridecanoyl glycerol	~99	3	0	0	555[b], 383	1.59

[a] Sigma Chemical Co., St. Louis, MO.
[b] Parent ion of molecular component.

was observed at lower wavelengths while still maintaining a reasonable response for each component. Fig. 1 shows the chromatogram of commercially available Imwitor 742 along with a blank injection showing the gradient and injection artifacts. Once a separation was obtained that eluted the desired number of peaks (based on Table 1) identification of those peaks was initiated. The peaks eluting between 15 and 20 min that are unlabeled are considered specific to the Imwitor 742 sample and are not thought to be MCG related.

Many of the peaks in the chromatogram (8 out of 16) could be readily identified by the use of available commercial sources of a given component (see Table 1). Additionally, identification of the various chain length monoglycerides, diglycerides, triglycerides and free fatty acids is also readily accomplished by LC/MS. For acylglycerols (under APCI conditions) the primary fragmentation occurs at the ester bond yielding fragments easily differentiated by the various chain lengths. These fragments, coupled with a strong parent ion signal allow for simple identification of the acylglycerol. For the free fatty acids, a strong parent ion can be expected, but increased energy will fragment the carbon chain at each methylene group [10]. For identification of the monoglyceride components a base peak corresponding to the molecular weight (*M*) along with a [M-18] peak corresponding to the neutral loss of water was observed. For the diglyceride components, peaks were observed corresponding to the [M], [M-18] and to [M-143 or 171], respectively. The peaks corresponding to [M-143] or [M-171] represented the fragmentation of the molecule with loss of the C8 (143) or C10 (171) side chains. For the triglyceride components, peaks were observed at the [M], [M-18] and at [M-143] or [M-171], respectively. Based on the approximate intensities of the [MW-143] and [MW-171] peaks, an assignment was made regarding the number of C8 and C10 chains on the molecule. Little fragmentation was observed for the free fatty acids and identification was accomplished using only the parent ion signal.

Identification of the 16 components labeled in Fig. 1 that are present in commercially available MCGs was accomplished by the combination of LC/MS and spiking with authentic mate-

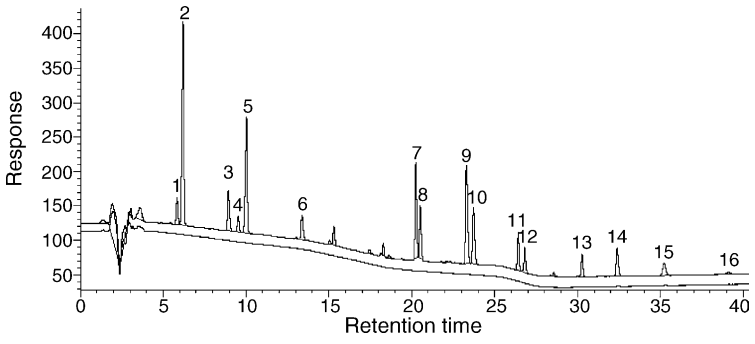

Fig. 1. HPLC-UV chromatogram of Imwitor 742. See Table 1 for peak assignments. Conditions given in Section 2.2.

Table 2
Linearity/RRF results for the HPLC-UV method

MCG component	Linearity		Relative response factor	
	Range (mg/mL)	R^2	wt.% basis	Molar basis
1-Mono-octanoyl glycerol	1.48–0.370	1.0000	1.11	1.26
1-Monodecanoyl glycerol	1.58–0.396	0.9999	1.00	1.00
Decanoic acid (free acid)	1.06×10^{-1} to 2.64×10^{-2}	0.9988	0.99	1.43
Octanoic acid (free acid)	9.64×10^{-2} to 2.41×10^{-2}	0.9994	1.20	2.07
1,2-Dioctanoyl glycerol	1.07–0.268	1.0000	1.48	1.07
1,2-Didecanoyl glycerol	1.07–0.266	1.0000	1.29	0.80
Tridecanoyl glycerol	$(0.451–2.41) \times 10^{-2}$	1.0000	1.59	0.71
Trioctanoyl glycerol	$(0.426–2.49) \times 10^{-2}$	0.9990	1.43	0.75

rial were available. This method not only provides separation of the main C8 and C10 components, but also provides partial resolution of the mono and diglyceride isomers. This additional selectivity provides further increased capability relative to the compendial GPC method available to most laboratories.

3.2. Method repeatability

The repeatability of the method was examined by performing 10 injections of the standard and evaluating the area response. The ten replicate injections yielded and R.S.D. of 0.6% indicating excellent injection precision of this method. The repeatability of sample preparation or standard preparation was not evaluated. In addition, multiple lots of sample were not readily available for analysis and examination of the lot to lot variability.

3.3. Relative response factor determination

To allow for accurate quantification of the full range of components in a MCG sample, the relative response factor (RRF = peak area of component/peak area of standard) for each peak was determined based on the standard, 1-monodecanoyl-rac-glycerol. The response factors were determined by generating a 3-point response curve and relating the slopes of the individual components to that of the standard at levels expected to be observed in commercial samples. Apparently, there is no clear preference (see Table 2) to use either a molar or weight based approach to quantitate the 16 components in Fig. 1, however a weight-based approach was used for all calculations in this paper.

The validated method can then be applied to examine commercial sources of acylglycerols. The first two examples were Capmul MCM and Imwitor 742, which were shown to contain very different ratios of C8 and C10 monoglyceride isomers, but similar ratios of C8 and C10 diglyceride isomers (see Table 3). This information may provide some insight into the functional performance aspects of the pharmaceutical formulation. It is interesting to note that these differences would not be observed if the two samples were analyzed by current compendial methods. Therefore, the RP-HPLC method provides an improved quality control measure of the medium chain glycerides.

To further illustrate the accuracy of the RP-HPLC method, a set of commercially available MCGs were analyzed by this new method and by a GC FAMES assay, which involved hydrolyzing the ester linkages and forming the methyl esters [18]. As illustrated in Table 4, the RP-HPLC method provides comparable results to the GC method; however the HPLC method provides a more robust sample preparation with comparable run time (40 min versus 45 min).

Table 3
Comparison of isomer ratios of Capmul MCM and Imwitor 742

Sample	1-C8:2-C8 ratio	1-C10:2-C10 ratio	1,3-C8:1,2-C8 ratio	1,3-C10:1,2-C10 ratio
Imwitor 742	12:1	12:1	2:1	2:1
Capmul MCM	17:1	17:1	2:1	2:1

Table 4
Results obtained via RP-HPLC method vs. results obtained via GC method (%)

	% C8 monoglycerides	% C10 monoglycerides	% C8 diglycerides	% C8–C10 diglycerides	% C10 diglycerides	% Total monoglycerides	% Total diglycerides	% Total triglycerides
Imwitor 742-GC	27.8	19.3	14.0	18.7	6.2	47.2	38.9	7.9
Imwitor 742-HPLC	28.0	19.5	14.7	18.8	6.2	47.5	39.7	7.7
Capmul MCM-GC	47.4	9.3	23.0	8.8	1.0	56.6	32.7	3.5
Capmul MCM-HPLC	46.8	9.0	24.0	8.9	0.9	55.9	33.7	4.0
Miglyol 812-GC	0.0	0.0	0.5	0.7	0.2	0.0	1.5	91.1
Miglyol 812-HPLC	0.0	0.3	0.3	0.8	3.2	0.3	4.2	95.5

4. Conclusion

A simple HPLC-UV method has been developed and validated to serve as a vital analytical tool for the full characterization of MCGs to be used in pharmaceutical formulations. This method utilizes quantitation by external standard to accurately quantitate individual components, total monoglycerides, total diglycerides, total triglycerides, total free fatty acids and caprylic versus caproic ratios. This method has been shown to be superior to current compendial methods, which offer only limited information regarding the ratios of mono-, di- and triglycerides present in the sample and eliminates the tedious sample derivitization techniques required to analyze the MCGs by GC-FID.

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