

# irm-LC/MS: $\delta^{13}\text{C}$ of Analgesic and Antipyretic Drugs

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## Key Words

- LC IsoLink™
- Aspirin®
- Compound Specific Isotope Analysis
- Drugs
- HPLC
- Isotope Ratio MS
- Paracetamol

## Introduction

Pharmaceuticals are produced in high amounts at various sites with different prices and different formulations. Therefore it is essential to the producers and consumers to distinguish the point of origin between originals and generics. Aspirin® and Paracetamol are representative for drug mass production with a wide range of origin and prices.

Acetylsalicylic acid introduced under the trademark Aspirin® in the year 1899 has a leading position in the prescription-free therapy of painful, inflammatory and feverish states. In 1971 the blood-thinning properties of acetylsalicylic acid, and then in 1991 its prophylactic effects against colonic cancer, were discovered.

Paracetamol or acetamidophenol, which was introduced in 1956, is known for its analgesic and antipyretic properties. It is also applied in formulations together with Aspirin and caffeine.

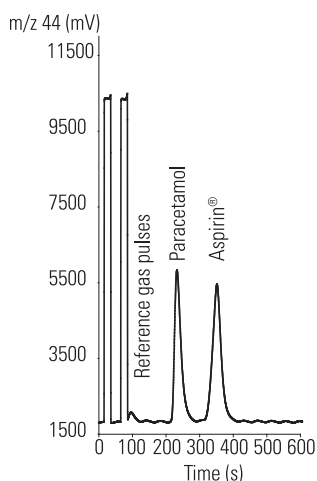


Figure 1: irm-LC/MS chromatogram of a tablet containing acetamidophenol and acetylsalicylic acid.

Isotope ratio monitoring-LC/MS (irm-LC/MS) with the Thermo Scientific LC IsoLink helps to determine the sources of pharmaceuticals via the individual  $^{13}\text{C}/^{12}\text{C}$  ratio ( $\delta^{13}\text{C}$  values) of the ingredients. Small amounts in complex mixtures can be applied for compound specific isotope analysis without extensive preparation or derivatization.

The  $\delta^{13}\text{C}$  value is the  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample related to the  $^{13}\text{C}/^{12}\text{C}$  ratio of a reference material to ensure international compatibility of data sets:  

$$\delta^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C})_{\text{Sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{Reference}} - 1) \times 1000$$
 For a rough estimation  $\delta^{13}\text{C}$  relates to atom% divided by 1000.



The isotope ratio of each compound can be used to reveal the history and hence the source of pharmaceuticals. E.g., acetylsalicylic acid is produced from phenol by carboxylation followed by acetylation with acetic anhydride. The origin of these substrates plus isotope shifts due to isotope fractionation in the reactions as it is known for the acetylation with acetic anhydride define the final  $\delta^{13}\text{C}$  value of the Aspirin. Similar effects are valid for all other compounds in such formulations.

The LC IsoLink adds a further new method to the analysis of pharmaceuticals. The bulk  $\delta^{13}\text{C}$  value ( $\mu\text{-EA}$ ) of all water soluble compounds in the tablet can be analyzed by injection of non-separated sub- $\mu\text{g}$  samples in aqueous solution. The ease of sample preparation, speed (< 100 sec/sample) and high sensitivity (factor 100 compared to EA) outperforms the classical elemental analyzer (EA) method by far.

## HPLC Parameters

Column:	Thermo Scientific HyPURITY AQUASTAR™
Eluent:	10 mM $\text{NaH}_2\text{PO}_4$
Flow Rate:	300 $\mu\text{L}/\text{min}$
Temperature:	50 °C
Loop Size:	5 $\mu\text{L}$
Sample Concentration:	100 ng/ $\mu\text{L}$
Oxidation Reagent:	0.88 M $\text{Na}_2\text{S}_2\text{O}_8$
Reagent Flow Rate:	30 $\mu\text{L}/\text{min}$
Reactor Temperature:	99.9 °C
He Flow:	1 mL/min

Table 1: HPLC and LC IsoLink parameters.

## irm-LC/MS Technology

The LC IsoLink is the first high sensitivity interface connecting high performance liquid chromatography (HPLC) with Isotope Ratio MS for the reproducible and accurate on-line determination of  $^{13}\text{C}/^{12}\text{C}$  isotope ratios. All organic compounds eluting from an HPLC column are analyzed while maintaining the chromatographic resolution.

In the LC IsoLink the sample is oxidized within the aqueous solvent eluting from the HPLC, afterwards the generated  $\text{CO}_2$  is separated from the liquid phase. This process is quantitative and fractionation-free.

The oxidation reagent consists of two solutions, the oxidizing agent and phosphoric acid. Both are pumped separately and added to the mobile LC phase. Within this mixture all individual organic compounds eluting from the HPLC column are oxidized quantitatively into  $\text{CO}_2$  when passing through a heated reactor. In a downstream separation unit the  $\text{CO}_2$  is removed from the liquid phase and entrenched into a stream of He. The individual  $\text{CO}_2$  peaks in He are subsequently dried in an on-line gas drying unit (Nafion<sup>®</sup>) and then admitted to the Isotope Ratio MS via an open split interface.

### Basic Sources of $\delta^{13}\text{C}$ Differences

Isotope ratio MS applications are based on the analysis of smallest isotope differences in compounds, originating from physical and biochemical isotope fractionation in nature. The isotope ratio differences of substrates mainly originate from two pathways (C3 and C4) of  $\text{CO}_2$  fixation in plants, which result in a  $^{13}\text{C}/^{12}\text{C}$  isotope ratio difference of approx. 15 ‰ ( $\delta$ -notation,  $\sim 0.015$  at%). Petroleum based compounds average around  $-30$  ‰.

This is already a wide range in isotope ratio MS in relation to the high precision of better  $\pm 0.2$  ‰. Chemical and physical processes in industrial production can enlarge this range.



Figure 2: Scheme of the Thermo Scientific *irm*-LC/MS system with the LC IsoLink.

### Calculation of amounts applied

Acetylsalicylic Acid (ASA) :  $\text{C}_9\text{H}_8\text{O}_4$   
Molecular weight = 180 ng/nmol  
Carbon content = 60 %

=> 500 ng ASA = 2.77 nmol ASA  
=> = 25 nmol carbon  
=> = 300 ng carbon

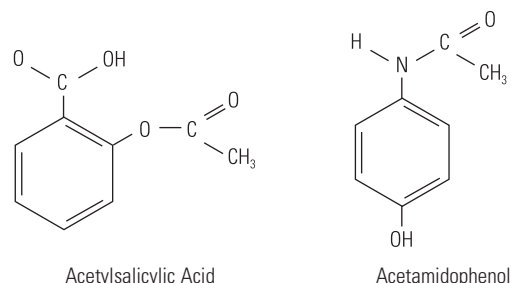


Figure 3: Structure of Acetylsalicylic Acid and Acetamidophenol.

### Determination of Sources and Differences in Pharmaceuticals

The ingredients of pharmaceuticals are set up by compounds from natural or industrial sources. Basic sources of carbon of these compounds or parts for building these compounds are petroleum, air, natural gas and plant materials.

The parts to build acetylsalicylic acid originate from petroleum and natural gas for the salicylic acid via benzene and phenol, and from natural gas for the acetic anhydride. Similar sources are used for paracetamol. Further additives can also originate from plant material like corn starch, which is quite commonly added in different amounts. Corn is a C4 plant with an isotope ratio of ca.  $-11$  ‰, which can shift the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio of tablets towards more positive  $\delta^{13}\text{C}$  values.

The combined  $\delta^{13}\text{C}$  values of tablets 1 and 3 indicate the same origin. Source 6 seems to use two different sources of aspirin for the production of 6a and, in combination with paracetamol, for 6b. Also tablet type 5 and 6a seem to be very similar but need more confirmation.

TABLET TYPE	$\delta^{13}\text{C}$ (‰) ASPIRIN	$\delta^{13}\text{C}$ (‰) PARACETAMOL	$\delta^{13}\text{C}$ (‰) TABLET BY $\mu$ -EA
1	-34.2		-33.4
2	-27.7		-27.6
3	-34.2		-33.5
4	-29.1		-27.6
5	-27.2		-26.6
6a	-26.8		-26.7
7	-32.6	-32.3	-31.7
6b	-33.8	-28.7	-31.3
8	-32.7	-29.2	-29.7
9		-30.7	-21.7

Table 2:  $\delta^{13}\text{C}$  values of tablets of different origin with Aspirin and Paracetamol measured by *irm*-LC/MS.

A comparison of the  $\delta^{13}\text{C}$  values of Aspirin in tablets of different origin with the  $\delta^{13}\text{C}$  values of the complete water soluble formulations of these tablets is shown in Figure 4. Three major sources of Aspirin can be detected (see circles). The additives in the different brands change the  $\delta^{13}\text{C}$  values of the tablets quite substantially. Thus the combination of both  $\delta^{13}\text{C}$  values allows further distinction between some of the sources.

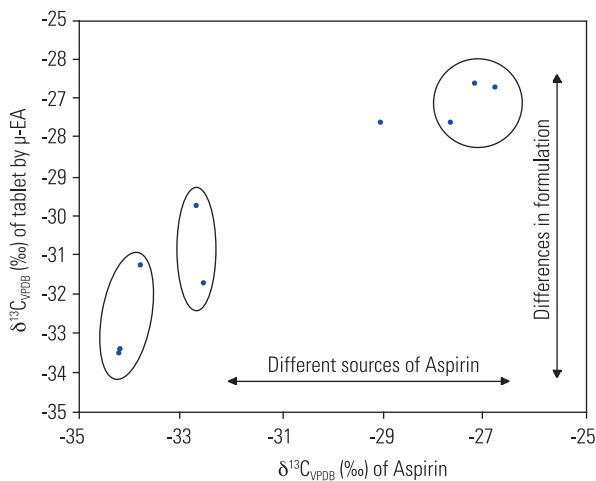


Figure 4: Comparison of  $\delta^{13}\text{C}$  values of Aspirin and tablet formulation.

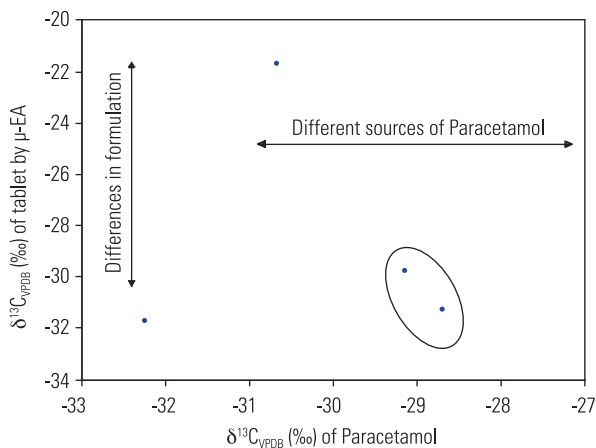


Figure 5: Comparison of  $\delta^{13}\text{C}$  values of Paracetamol and tablet formulation.

The comparison of  $\delta^{13}\text{C}$  values of paracetamol and whole tablets is shown in Figure 5. Tablet 6b and 8 might use the same source of paracetamol but need more confirmation. All four tablets are used in different formulation. Tablet 9 is a formulation without Aspirin (see Figure 6).

### $\mu\text{-EA}$ by Flow Injection

The LC IsoLink offers the fast analysis of bulk samples using its direct loop injector positioned immediately after the HPLC column. The bulk samples are processed exactly the same way as the HPLC separated compound.

This micro elemental analyzer ( $\mu\text{-EA}$ ) method can be used for fast bulk analysis of sub- $\mu\text{g}$  samples in aqueous solution. An advantage of this method is the direct comparison with reference samples of a certified  $\delta^{13}\text{C}$  value. The ease of sample preparation, speed (< 100 sec/sample) and high sensitivity (factor 100 compared to EA) outperforms the classical EA method by far.

Co-injection of the bulk aqueous solution of the tablet can also be used within every HPLC run. The injection at the beginning or at the end of the HPLC chromatogram speeds up analysis with almost no loss in analysis time.

Figure 6 shows the different compounds eluted from the reversed phase column followed by direct loop injection of the water soluble parts of the tablet. The comparison of the  $\delta^{13}\text{C}$  values of the eluted compounds and the bulk value explains the strong shift to more positive  $\delta^{13}\text{C}$  values in the bulk injection. It also shows that there is more material, which can not be eluted, with more positive  $\delta^{13}\text{C}$  values.

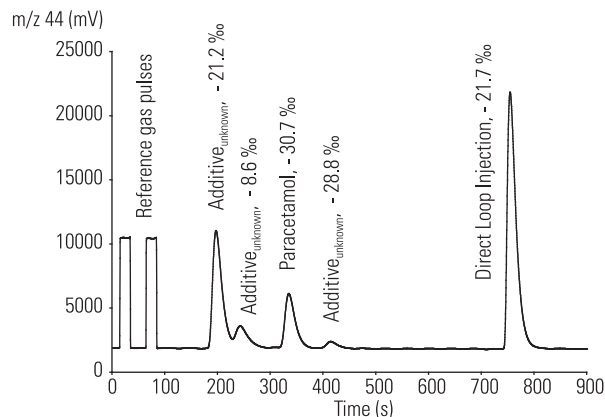


Figure 6: Analysis of tablet 9 followed by direct loop injection ( $\mu\text{-EA}$ ). Loop size of the HPLC injector was  $5\ \mu\text{L}$ , the loop size of the  $\mu\text{-EA}$  injector was  $10\ \mu\text{L}$ , which results in two-fold response of the  $\mu\text{-EA}$  peak.

### References

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