DETERMINATION OF VITAMIN A IN FORTIFIED COOKIES

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Fortification of different foods is very common nowadays. Our country is aligned with this trend and is developing a lot of fortified products. One of these new products is vitamin A fortified cookies. We worked in a sensitive, accurate, precise and fast normal phase HPLC-FLD to help the industry to control the fortification process.

EQUIPMENT:

HPLC Agilent 1100 with:
Fluorescence Detector G1321A operating at 325-475 nm
Diode Array Detector G1315B operating at 325 nm
Automatic Injector Agilent 1313A
Column: Phenomenex Luna Si (2) 100A 25cm x 4,6mm x 5u

EXPERIMENTAL:

a- Sample is dispersed in ethanol and saponificated with aqueous KOH.To spiked samples, the all-trans-retinyl acetate in ethanol is added before the KOH addition.
Two extractions are performed with a mixture 75:25 hexane:ethyl ether. The organic layers are washed with water.
HPLC analysis
mobile phase: hexane with 3% isopropyl alcohol
flow: 2 ml/min
oven temperature: 25 °C
b- We tested the same procedure with the addition of takadiastase:
enzime treatment prior to saponification: 0,1 g of takadiastase is used for 1 g of sample. The sample is added to the enzyme aqueous solution. After enzyme treatment, the same procedure described above is followed.

Note: all-trans-retinyl acetate and all-trans-retinol standards used in this work, were tested for purity with UV-VIS spectrometer with quartz cells of 1 cm path length. Further checks on these standards are performed calculating three different ratios of values of absorbances measured at four different wavelengths.

VALIDATION REPORT:

ACCURACY: we use as a measure of accuracy the recovery from spiked blank samples (the same cookies with no added vit A).

RECOVERY: we spiked the blank sample at different levels with all-trans-retinyl acetate solution in ethanol

Level 1: 4 ug/g of retinol recovery: 102% (n=3 RSD=1.9%)
Level 2A (target level): 10.5 ug/g of retinol recovery: 98% (n=3 RSD=0,4%)
Level 2B (target level): 13 ug/g of retinol recovery: 85% (n=3 RSD=2,1%)
Level 3: 26 ug/g recovery: 86 % (n= 3 RSD= 0.8%)

In order to confirm that two extractions are enough for good recovery we spiked two blank samples with all-trans-retinyl acetate solution equivalent to 33 ug retinol (higher than the target level), and we analyzed both extractions separately. The recovery in the second fraction was 11 % of the total amount.

Note: For level 1 and 2A we used an all-trans-retinyl acetate powder lot A and for level 2B and 3 the all-trans-retinyl acetate powder was lot B. Lot B has a wrong value for one of the absorbance ratios measured, and lot A has correct values for all the ratios.

PRECISION:
RSDr: ranged from 0.4 to 1.9 % (n=3)
iRSDR: 4% (n=3)

CALIBRATION CURVE/LINEARITY:

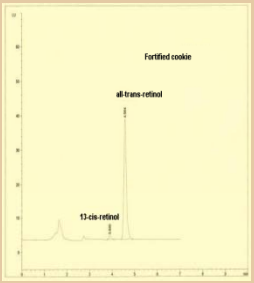
FLD: 0.02 ng -95 ng (more than 15 levels) r=0,9999
DAD: 0.2 ng -18 ng (9 levels, not tested for higher values) r=0,9999

DETECTION LIMIT:
we consider as the detection limit the lowest detectable amount. This signal must be bigger than a signal /noise ratio of three.
FLD: 0.01 ng in column (0.003 ug/g cookies)
DAD: 0.1 ng in column

QUANTITATION LIMIT: we consider as the quantitation limit the lowest point of the calibration curve.
FLD: 0.02 ng in column
DAD: 0.2 ng in column

SPECIFICITY: in the conditions described above, we obtained a very good peak of all-trans-retinol with no interferences. As a routine we use to work with FLD because is selective and more sensitive than DAD. We tested the DAD too in order to use UV spectra for identity confirmation.

SENSITIVITY: we can quantificate levels higher than 0.02 ng in column using FLD.



RUGGEDNESS:

a- Enzyme treatment is not necessary. We performed this treatment in a spiked sample with 13 ug retinol. The recovery is the same for this level if we add enzyme or not.
b- We tested as a measure of ruggedness the stability of the sample solutions stored at -18°C. We injected them immediately after the extraction and 6 and 10 days after. The extractions were stable during this period.

UNCERTAINTY: 7 %

CONCLUSIONS:

*Due to the results obtained with or without enzyme treatment we concluded that enzyme addition is not necessary. The recovery is the same, and we did not notice any improvement during the extraction procedure. The chromatograms are almost identical. *The recoveries obtained after the fortification with the all-trans-retinyl acetate powder with one wrong value of absorbance ratios, are smaller than the recoveries obtained after using the all-trans-retinyl acetate powder with all the absorbance ratios with correct values. This shows this additional check for purity using absorbance ratios is an important issue to consider.
*We performed the validation with FLD, but DAD could be used. No interferences in chromatograms using DAD were noticed.
*The recovery in the second extraction was 11%, even in a higher level than the target level .We concluded that two extractions are enough for good recovery.
*13-cis-retinol is only a small fraction of the total amount of vitamin A, so we quantified only the all-trans-retinol isomer.
*The analysis presented here is sensitive, accurate, precise and useful for routine analysis.