Purification of oligonucleotide using continuous chromatography (MCSGP)

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Introduction

An efficient purification is required in downstream process for oligonucleotide pharmaceuticals due to related impurities having similar chemical properties. While a conventional single-column purification faces a purity-yield trade-off challenge, a continuous chromatography process: MCSGP (**M**ulti-column **C**ounter-current **S**olvent **G**radient **P**urification) achieves both high purity and yield at the same time by recycling side-cut portions periodically. In this MCSGP study using Contichrom® CUBE, yield was improved 1.3-fold compared to its single column purification. The purification time and required amount of crude were estimated in scale-up simulation study.

Chontichrom® CUBE

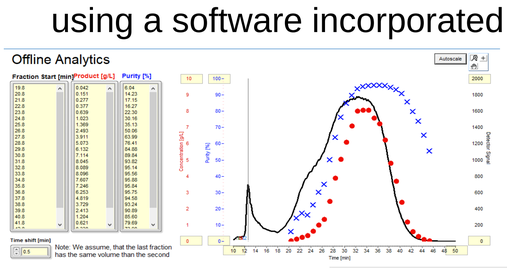
▼ Sample

Classification : Crude Model MOE-ASO 18mer, PS(full), 2'-MOE
Sequence : 5'-TCACTTTTCATAATGCTGG-3'
Purity : 75.6% or 70.2%

▼ Purification

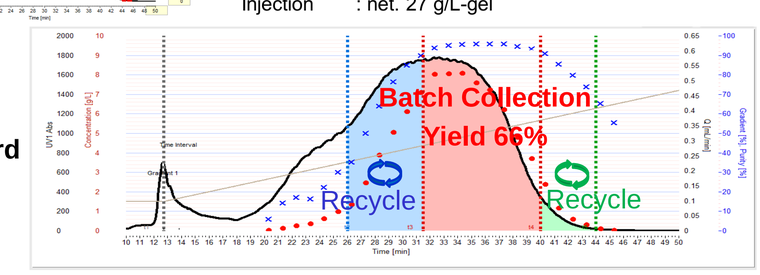
Batch :Single Column Chromatography

✔ MCSGP condition can be configurated from Batch fraction data using a software incorporated in Contichrom® CUBE.



Column : BioPro IEX SmartSep Q (20 µm) 100 X 5 mmI.D.
Eluent : A) 20 mM Tris-HCl (pH 8)
B) 20 mM Tris-HCl (pH 8) containing 3M NaCl
15-75%B (12 CV)
Flow rate : 0.6 mL/min
Temperature : ambient
Detection : UV at 290 nm
Injection : net. 27 g/L-gel

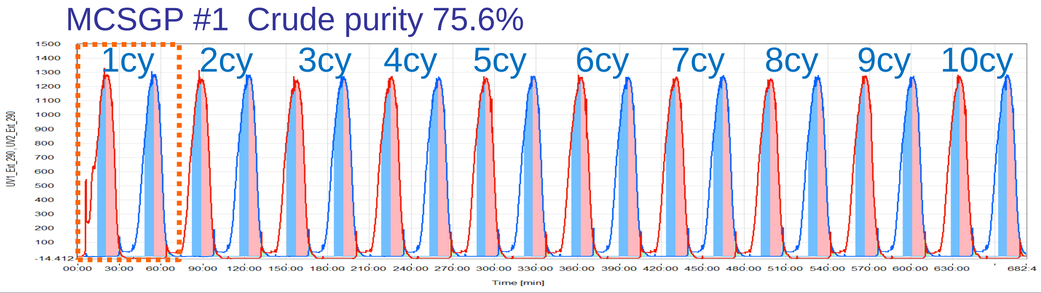
Guided procedure through MCSGP wizard



MCSGP: Continuous Chromatography

✔10cy-MCSGP was successfully conducted with keeping purity criterion ≥93%, regardless of crude purity.
✔After 2nd cycle, the product of each cycle was constantly obtained in high yield, 85-90%.

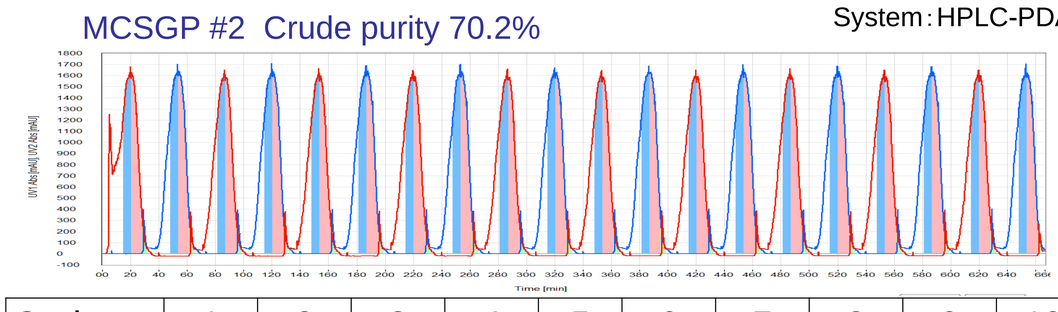
MCSGP #1 Crude purity 75.6%



Cycle	1	2	3	4	5	6	7	8	9	10
Purity %	94.9	93.3	93.1	93.0	93.1	93.1	93.0	92.8	92.7	93.0
Yield %	72.7	88.6	88.0	88.0	89.4	89.5	89.4	89.5	88.0	88.5

System : HPLC-PDA

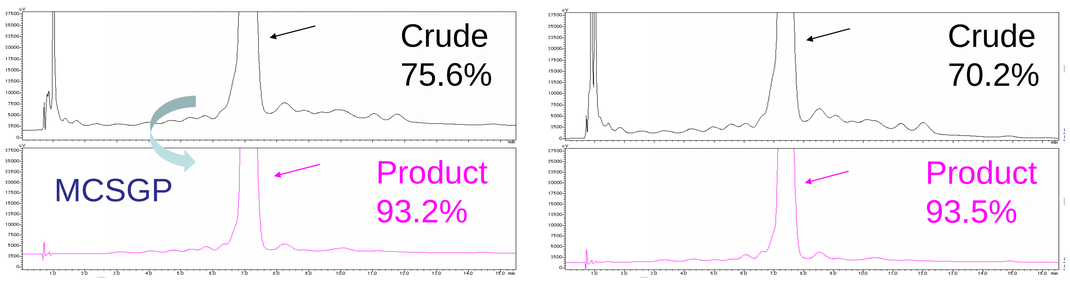
MCSGP #2 Crude purity 70.2%



Cycle	1	2	3	4	5	6	7	8	9	10
Purity %	94.2	93.5	93.4	93.4	93.4	93.4	93.3	93.3	93.3	93.4
Yield %	70.8	85.7	85.9	85.7	85.6	85.5	85.7	85.4	84.9	85.5

System : HPLC-PDA

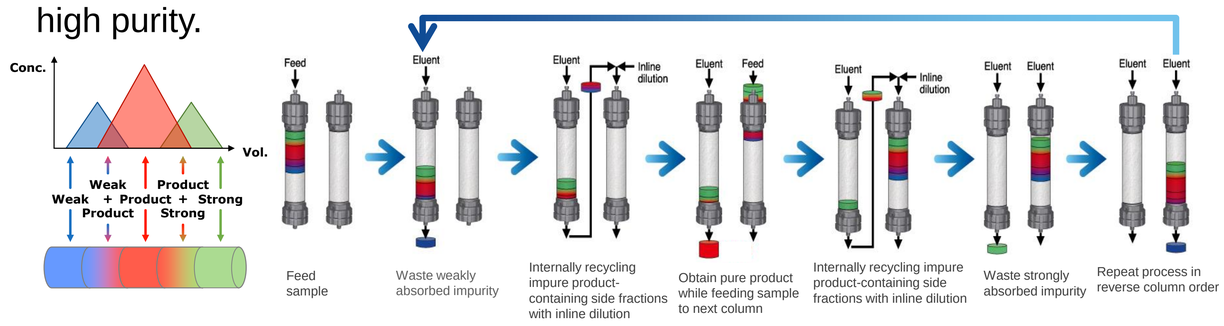
▼ Quality



Column : Accura Triart Bio C18 (1.9 µm) 50 X 2.1 mmI.D.
Eluent : A) 15 mM TEA-400 mM HFIP
B) methanol
22-40%B (0-18 min)
Flow rate : 0.2 mL/min, Temperature : 60 ℃, Detection : UV at 260 nm

▼ MCSGP

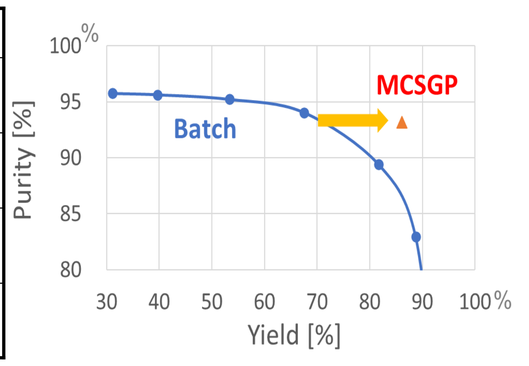
MCSGP is a purification process which two columns are used in continuously. Feed sample is separated through a column by solvent gradient to pure product (red), impurities (blue and green) and mixture of impurities/product (blue/red and red/green). In a conventional single-column purification, the mixtures which are not pure enough are wasted or collected for re-chromatography. On the other hand, MCSGP recycles these portions to the other column with in-line dilution, enabling to prevent the loss of precious target compound. In addition to recycled portions, new feed is injected with the same amount of pure product collected in the previous column. Repeating this cycle achieves high yield with keeping high purity.



▼ Comparison with Batch

✔ The yield was improved by 1.3-fold compared to Batch.
✔ The trade-off between purity and recovery was overcome.

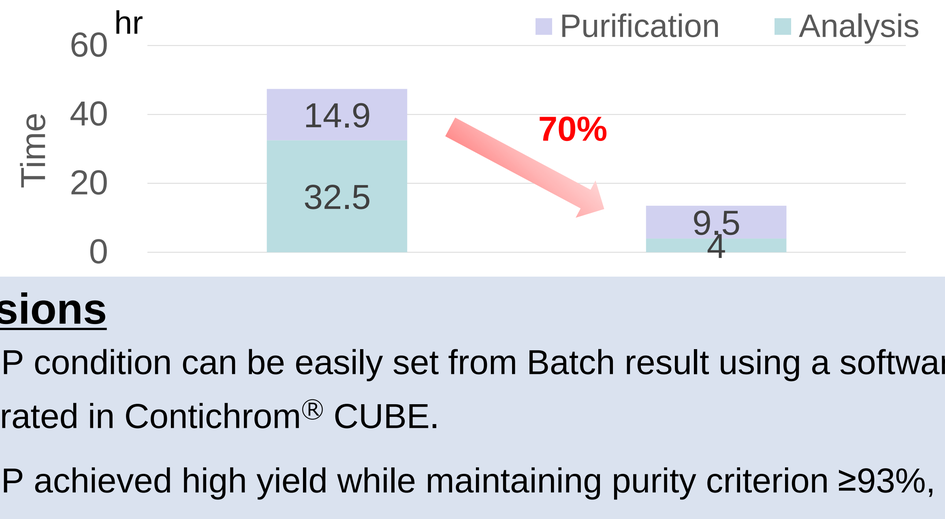
	Batch		MCSGP #1	MCSGP #2
CrudePurity %	75.6		75.6	70.2
Product Purity %	94.0		93.2	93.5
Yield %	66	X 1.3	86	83
Productivity mg/hr	33.5	X 1.6	52.4	52.0



▼ Scale-up simulation: 200 g production

✔Overall purification time will be cut 70% from Batch process.
✔Synthesis time and cost can be saved due to the decreased amount of crude.

	Batch		MCSGP
Crude	301 g		232 g
Column size	100 X 100 mmI.D. (resin vol: 0.79 L)		100 X 100 mmI.D. x 2 (resin vol: 1.57 L)
Purification MC time	14.9 hr (13 run)		9.5 hr (8 cycle)
Purification QC time	32.5 hr		4 hr
Purification Total time	**47.4 hr**		**13.5 hr**



Conclusions

- MCSGP condition can be easily set from Batch result using a software incorporated in Contichrom® CUBE.
- MCSGP achieved high yield while maintaining purity criterion ≥93%, regardless of crude purity. The yield was improved by 1.3-fold compared to Batch. A purity-yield trade-off was overcome.
- In scale-up simulation study, MCSGP reduces purification time by 70% and can save the cost due to decreased amount of crude material.

Acknowledgment

✔This poster was supported by AMED under Grant Number JP21ae0121022, JP21ae0121023, (Project leader: Satoshi Obika).
✔A model antisense oligonucleotide ("Crude Model MOE-ASO 18mer") was synthetized by KNC Laboratories Co., Ltd.

KNC Laboratories Co., Ltd.

Conflict of interest (COI)

We have no financial relationships to disclose for this presentation.